Final Report

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Volatile Fatty Acids, Amine, Phenol, and Alcohol Emissions from Dairy Cows and Fresh Waste

Submitted to: California Air Resources Board

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CONTENT TABLE

1 EXECUTIVE SUMMARY

Volatile fatty acids, phenols, amines, and alcohols were measured from dry and lactating Holstein cows and their waste in an environmental chamber. The first period with cows in the chamber showed emissions from enteric fermentation. On day one of each experimental period, waste accumulated on the floor over 24 hrs and remained in the chamber for a second day to allow for measurements of 'cows only', 'cows and waste', and 'waste only'. Seven airborne volatile fatty acids (VFAs) and five phenolic compounds emitted from dairy cows and their wastes were sampled using sorbent tubes and later analyzed on a thermal desorption TDS/GC-MS system at the National Soil Tilth Laboratory in Ames, Iowa. Amines were sampled using acid impingers and analyzed using ion chromatography at UC Davis. Alcohols and methane were measured in real time using two photoacoustic INNOVA gas analyzers. Complete information of chamber temperature, humidity, and air flow were collected during all testing periods.

The main findings can be summarized as follows:

1. Methane was produced at high fluxes from both dry and lactating cows and at very low fluxes from fresh waste. Methane was associated with enteric fermentation of dairy cows.

2. VFAs and phenols were detected to a lesser degree from cows than from fresh waste. However, both VFA and phenol concentrations measured were at the lower detection limit of the assay and instrumentation.

3. None of the amines were detected from cows and waste at a concentration level of 10 ppb or higher in the exhaust air from the chamber.

4. Alcohols were measured at high concentrations. Both ethanol (EtOH) and methanol (MeOH) were produced during enteric fermentation (eructated gas). However, both EtOH and MeOH increased over time in correspondence with accumulating fresh waste. With increasing residence time of cows and waste in the chamber, both alcohols increased continuously to reach the highest levels after 24 hours. After cows were removed from the chamber (on the second day of each period), both alcohols remained at high fluxes for several hours but then decreased over time.

2 INTRODUCTION

In August 2005, the San Joaquin Valley Air Pollution Control District (SJVAPCD) released the new emission estimate for volatile organic compounds (VOC) from dairies. This new 19.3 lb dairy emission factor report lists a subgroup of the VOCs, namely volatile fatty acids (VFAs), as the main reactive gas fraction of VOCs, totaling 15.5 lbs/cow/yr. The portion assigned to enteric VFA emissions (8.3 lb/cow/yr) was based on an extremely limited data set. Aside from VFA, estimates of phenolic compounds, amines, and alcohols were uncertain and warranted further investigation.

3 OBJECTIVE

To quantify volatile fatty acid (VFA), amine, phenol, and alcohol emissions from cows (enteric fermentation) and their fresh waste under controlled conditions simulating dairy freestall housing.

4 MATERIAL AND METHODS

Environmental chambers

The study was conducted in a newly constructed environmental chamber at the University of California, Davis. The environmental chamber (5,000 cft volume) has a continuous air exchange of 1,320 cfm, which provides a controlled environment providing consistent conditions. All UC Davis animal facilities are certified by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and the Institutional Animal Care and Use Committee (IACUC) approved the project to certify the health and safety of animals. Additionally, the environmental chamber has been certified for consistent air flow and ambient temperature control capabilities by the engineering department of UC Davis.

The environmental chamber has one incoming and one outgoing airduct. Air samples were obtained in the center of the incoming and outgoing airducts immediately above the chamber ceiling. This ensured that the inside chamber conditions remained undisturbed (no need to open chambers) during the actual measurements. Atmospheric measurements of the empty chamber on the first day of each sampling period were conducted to assess background VOC concentrations. Temperature, humidity, and airflow in the chamber were continuously measured and recorded. Temperature was held constant during all sampling events at 65° Fahrenheit and air flow at 1,320 cfm.

Animals

Experiment 1 (Exp. 1):

During each test run (period) in Exp. 1, three cows were placed in the environmental chamber for testing. A total of six test runs of three cows were performed. Three of the six test runs were performed with dry cows and three were performed with lactating cows. Emphasis of the Exp. 1 test runs were VFA, phenol, and amine emission. Alcohol emissions were not measured during Exp. 1.

Experiment 2 (Exp. 2):

To investigate alcohol Exp. 2 was conducted with additional cow groups. During each test run (period) in Exp. 2, three cows were placed in the environmental chamber for testing. A total of

seven test runs of three cows were performed. Four of the six test runs were performed with dry cows and three were performed with lactating cows.

Cows were fed a total mixed ration diet, formulated to meet 2001 National Research Council (NRC) nutrient requirements for either dry or lactating cows, respectively. Feed ration and cow performance are shown in table 1.

Table 1: Feed ration and performance for dry and lactating cows.

Ingredient	***Dry Cows***	***Lactating Cows***
Grain	0	34.8
Alfalfa	31.0	39.2
Oat Hay	61.0	0
Whole Cottonseed Meal	0	11.3
Almond Hulls	0	8.1
Soybean Meal	0	4.0
Milk Mineral	0	1.6
Energy II	0	0.6
Salt	0	0.3
Dry Cow Pellet	8.0	0
Total DM Ingested (DMI; kg cow^{-1} day^{-1})	7.6	9.1
Average Milk Yield (kg cow^{-1} day^{-1})	0	31.6
Average Body Weight (kg cow^{-1})	747.3	618.3

General

During Exp. 1, a total of 756 atmospheric sorbent tube and impinger samples were collected (252 samples per VFA, phenol, and amine assay each (see table 2, 3 and 4)) as well as continuous samples of alcohols and methane in Exp. 2.

Table 2 shows the sampling regimen. Sampling for each cow group includes initial sampling of the “empty chamber”, periodic sampling with “cows only” (no waste), with “cows and waste”, and

with “waste only” in the chamber, as well as 100% duplicate samples for quality assurance, in-chamber ‘grab samples’, and field blanks. Additional details are shown in Tables 3 and 4. Grab samples were obtained inside the environmental chamber at approx 3ft height.

Table 2: Summary of atmospheric sample numbers for volatile fatty acids (VFA), phenols (P), and amines (A) for six cow groups in Exp 1. Alcohols and methane in Exp. 2 were measured continuously.

Phase	Cow groups	Days	Inlet (# of samples)	Outlet (# of samples)
Dry cows	Group 1*	2	23 VFA[1]; 23 P[2]; 23 A[3]	23 VFA; 23 P; 23 A
	Group 2	2	23 VFA; 23 P; 23 A	23 VFA; 23 P; 23 A
	Group 3	2	23 VFA; 23 P; 23 A	23 VFA; 23 P; 23 A
Lactating cows	Group 4	2	19 VFA; 19 P; 19 A	19 VFA; 19 P; 19 A
	Group 5	2	19 VFA; 19 P; 19 A	19 VFA; 19 P; 19 A
	Group 6	2	19 VFA; 19 P; 19 A	19 VFA; 19 P; 19 A
TOTAL			126 VFA; 126 P; 126 A	126 VFA; 126 P; 126 A

* = each group consists of three cows
[1] VFA = Volatile Fatty Acids
[2] P = Phenols and cresols
[3] A = Amines

Schedule of Events within Experiment

In Exp. 1, six different groups of cows (three cows per group) were used to measure gases produced by animals (during enteric fermentation) and their fresh waste. Overall, on the first day of each measurement period (and prior to introducing cows to chambers), the inlet and outlet air (Photo 8) of the empty chamber was measured for VFAs, phenols, and amines. After two hours of 'empty chamber' measurement, three cows were placed inside the chamber to measure VFA, amine, and phenol concentrations in the air inlet and outlet airducts. On the second day, cows were removed while their waste remained on the chamber floor. Again, VFA, amine, and phenol emissions were measured in the air inlet and outlet airducts. The first three Exp. 1 groups were dry cows (three replications; n = 3), and the second three groups were lactating cows, measured within subsequent weeks (see table 3 and 4 for details). Exp. 1 dry cows stayed in the chamber for 24 hrs and lactating cows for 9 hrs periods. Exp. 2 was conducted to continuously measure methane and alcohol emissions using two photoacoustic gas analyzers (INNOVA). In Exp. 2, both dry cow and lactating cow groups stayed inside the chambers for 24 hrs and lactating cows were milked at 8 a.m. and 7 p.m. Milking was performed by the UC Davis dairy staff inside the chamber using a mobile milking machine. During 30 min around theses milking events, chambers were opened and closed once.

Table 3: Summary of sampling schedule and sample numbers for Exp. 1 for one replication of dry cows (Note: Exp 1. had three replications = three dry cow groups).

	DRY COWS AND WASTE (one cow group of 3)									
Chamber occupancy	**Empty chamber**	**Cows Only**	**Cows & waste**	**Cows & waste**	**Cows & waste**	**Cows & waste**	**Waste**	**Waste**	**Waste**	**Waste**
Sampling time	Day 1 4:30-6:30 -a.m.	Day 1 7-9 a.m.	Day 1 2-4 p.m.	Day 1 8-10 p.m.	Day 2 2-4 a.m.	Day 2 7-9 a.m.	Day 2 2-4 p.m.	Day 2 8-10 p.m.	Day 3 2-4 a.m.	Day 3 7-9 a.m.
Inlet	2 sample*	2 samples	2 sample	2 sample	2 sample	3 samples	2 sample	2 sample	2 sample	4 samples
Outlet	2 sample	2 samples	2 sample	2 sample	2 sample	3 samples	2 sample	2 sample	2 sample	4 samples
Comment	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate & Grab samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate & Grab sample & Field blank*

1 sample stands for 1 VFA sample, & 1 phenol sample, & 1 amine sample

Grab samples will only be conducted for one set of dry cows.

Table 4: Summary of sampling schedule and sample numbers for one replication of lactating cows in Exp. 1. (Note: Exp. 1 had three replications = three lactating cow groups).

	EXP 1. LACTATING COWS AND WASTE (one cow group of 3)							
Chamber occupancy	**Empty chamber**	**Cows Only**	**Cows & waste**	**Cows & waste**	**Waste**	**Waste**	**Waste**	**Waste**
Sampling time	Day 1 4:30-6:30 a.m.	Day 1 7-9 a.m.	Day 1 11 am-1 p.m.	Day 1 3-5 p.m.	Day 1 7-9 p.m.	Day 2 3-5 a.m.	Day 2 11 am - 1p.m.	Day 2 7-9 p.m.
Inlet	2 sample*	2 samples	2 sample	3 samples	2 sample	2 sample	2 sample	4 samples
Outlet	2 sample	2 samples	2 sample	3 samples	2 sample	2 sample	2 sample	4 samples
Comment	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate & Grab samples***	*Duplicate samples*	*Duplicate samples*	*Duplicate samples*	*Duplicate & Grab samples** & Field blank*

* *1 sample* stands for 1 VFA sample & 1 phenols sample & 1 amine sample

** Grab samples will only be conducted for one set of lactating cows.

Note: The measurement schedules between dry and lactating cow trials differed for Exp. 1. Lactating cows remained in the chamber for 9 hr periods due to the necessity of being milked twice a day. Dry cows were returned to the University dairy after 24 hrs and lactating cows after 9 hrs of measurements. While atmospheric sampling for dry cows were conducted every 6 hrs (over 24 hrs), lactating cows were measured in 3 hr intervals over 9 hrs of total residence time in the chamber.
In Exp 2. lactating cows remained in the chamber for 24 hrs and air was sampled continuously. Animals were milked inside the chamber at 8 a.m. and 7 p.m. Milking was performed by the UC Davis dairy staff inside the chamber using a mobile milking machine. During 30 min around theses milking events, chambers were opened and closed once.

Photo 1: Empty environmental chamber.

Photo 2: Cows in chamber (no waste).

Photo 3: Space above chamber. All analyzers are attached to the chamber air inlet and outlet manifolds.

Photo 4: Innova 1412 measuring alcohols and methane.

Photo 5: Gerstel autosampler (containing sorbent tubes) and impinger train.

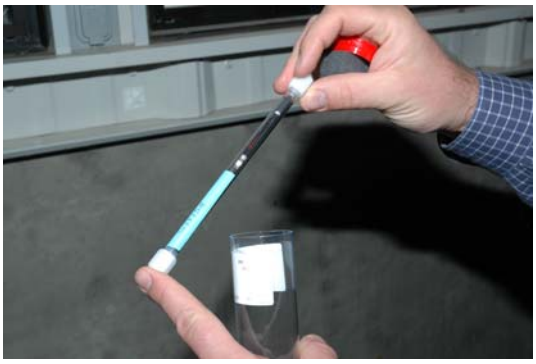

Photo 6: Sorbent tube for VFA & phenol.

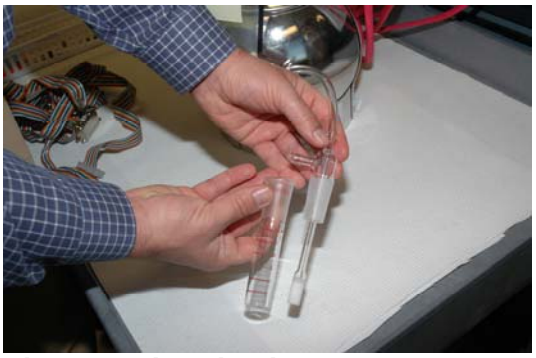

Photo 7: Glass impinger (containing acid to trap amines).

Photo 6: Ion chromatography (amine analyzer).

Photo 8: Chamber ceiling showing air inlet and air outlet. Air flaps within the inlet and outlet are directed in opposite directions to ensure chamber air mixing.

Sampling and Analytical Methods

Table 5 summarizes the sampling and analytical methods.

Table 5: Summary of analytical species, methods, sampling media, instrumentation and laboratories used for analysis.

Target Species	*Analytical Method*	*Sample Media*	*Analytical Instrument*	*Laboratory*
VFAs	Modified USEPA Method TO-17	Carbopack C & Carbopack X	GC/MS thermodesorbtion	USDA-ARS, Nat. Soil Tilth Lab
Phenols	Modified USEPA Method TO-17	Carbopack C & Carbopack X	GC/MS thermodesorbtion	USDA-ARS, Nat. Soil Tilth Lab
Amines	NIOSH 2010/SCAQMD 207.1	0.1N H2SO4 Impinger	GC/IC	Mitloehner lab at UC Davis
Alcohols	Photoacoustic	In situ	INNOVA 1412	Mitloehner lab at UC Davis

Volatile Fatty Acids (VFA)

The following **volatile fatty acids** were measured:

- Acetic acid
- Propionic acid
- Isobutyric acid
- Butyric acid
- Isovaleric acid
- Valeric acid
- Hexanoic acid

Phenols and cresols

The following **phenols and cresols** were measured:

- Phenol
- 2-methylphenol (o-cresol)
- 3-methyl phenol (m-cresol)
- 4-methylphenol (p-cresol)
- 4-ethylphenol Phenol

Air sampling for VFAs and phenolic compounds was conducted at the inlet and outlet air ducts of the animal chamber using sorbent tubes along with periodic grab samples from within the animal chamber. Four DESAGA gas samplers (model number GS-301) were used to accomplish a sequential sampling over six, two day time periods. The sampling intervals during which animals occupied the chamber were 6 (Table 3), and 3 hours (Table 4) for dry and lactating cows, respectively, while sampling intervals from chambers with waste alone were 6 and 8 hours, respectively.

All VFA and phenol air samples were collected in glass multi-bed thermal desorption tubes. Analysis was conducted using a Gerstel TDSA (Gerstel, Inc.) interfaced with Agilent 68126/5973N GC-MS (Agilent, Inc. Wilmington, DE). Further details regarding sampling, analysis, validation and QA/QC are described in the appendix.

Alcohols

The following **alcohols** were measured:

- Ethanol
- Methanol

Ethanol and methanol were analyzed continuously, using two INNOVA photoacoustic Field Gas-Monitors (Model 1412 and Model 1312; http://www.innova.dk/1412_details.gas_monitoring4.0.html). These instruments have a linear response over a wide dynamic range and high stability, which makes calibration necessary only a few times a year. However, for the present experiments, the instruments were factory calibrated in monthly intervals. The INNOVA 1412 measured EtOH and MeOH individually. The INNOVA 1312 measured total alcohols. INNOVA gas analyzers are EPA approved reference instruments for alcohol measurements. They are considered as a highly accurate, reliable and stable quantitative gas monitoring system based on the photoacoustic infra-red detection method. These instruments can measure almost any gas, which absorbs infra-red light. In addition to the two alcohols, these instruments also analyzed methane concentrations.

Amines

The following **amines** were measured:

- Dimethylamine
- Ethylamine
- Trimethylamine

- Isoprophylamine
- Propylamine
- Butylamine

Air samples of airborne amines emitted during dairy cow enteric fermentation were collected from inlet and outlet chamber air manifolds using impinger sampling trains (containing four impingers per train). Impingers contained sulfuric acid, which trapped amines once the air was bubbled through. Impinger samples were analyzed in the Mitloehner lab at UC Davis using ion chromatography (Dionex DX500; Dionex, Sunnyvale, CA) following a modified SCAQMD 207.1 protocol. A detailed amine SOP for sampling and analysis as well as QA/QC can be found in the appendix.

Quality Control and Chain of Custody

Duplicate samples were taken at both the inlet and outlet air ducts for all VFA, phenol, amine, and alcohol sampling events. Sampling time for all tube and impinger samples was 2 hrs (per sample interval). For quality control, 5% of sorbent tubes and impingers were opened to ambient conditions to allow for passive diffusion to occur and these samples were used as field blanks (see table 3 and 4). Additional 5% of sorbent tubes and impingers were used as lab blanks. Spike recovery was conducted for all compounds tested in the present experiment. The INNOVA analyzers were calibrated in four week intervals by the manufacturer.
Finally, every sample (sorbent tube and/or impinger) was accompanied by a chain of custody form (CCF). Samples were assigned sequential identification numbers. These numbers were logged and noted on the sample tags and documented (date, time, etc.) on the CCF. The CCF accompanied the samples to the analytical laboratory where the identical information was assigned to the results. Further QA/QC information can be obtained in the appendix.

5 RESULTS

Upon entry of both dry and lactating cows into chambers, **methane** fluxes (Table 6) immediately increased indicating that enteric fermentation is the main process in the formation of this gas. After removal of cows from chambers (but with waste present), methane flux went back to background levels. Values listed as '0' in Table 7 and elsewhere in the report were below the LOQ for the method. The term 'N/A' is used when valid data were not available for the test point.

The only **volatile fatty acid** (VFA) consistently above its Limit of Quantification (LOQ) was acetic acid. Butyric acid was typically above the method LOQ during at least one sampling event per replicate. On an emission mass basis, acetic acid contributed between 12-100% of total VFA emissions (Table 7). However, it should be noted that on the first dry cow sampling, the lowest calibration standard used for creating the calibration curve was higher than the highest value determined on the sorbent tubes collected from the chamber. Consequently, for the first dry cow sampling, the calibration curve used was extrapolated below the lowest available calibration standard. Subsequently, calibration curves were developed such that the levels of VFAs and phenolic compounds detected on sorbent tubes were consistently bracketed by the calibration standards. The use of an extrapolated calibration curve may explain why concentrations of compounds in the first dry cow group were higher than subsequent replicates, which used calibration curves that bracketed actual sample concentrations. If the first dry cow group was removed, acetic acid would still remain the main VFA, but its contribution would change to between 32-100% of total VFA emission. Grab samples taken within the environmental chambers demonstrate that acetic acid was the major VFA with low to non-quantifiable levels measured for all other VFAs. Field blank samples had no quantifiable levels of any of the target VFA compounds.

All **phenolic compounds** were typically above method LOQ for outlet air samples, while inlet air samples were typically below method LOQ. On an emission mass basis, 4-methylphenol was the most significant phenolic compound calculated at 40-50% of the total emission for the phenolic compounds. Other than 4-methylphenol, the most significant phenolic compounds were phenol, 2-methylphenol and 2-ethylphenol (Table 8). Grab samples taken within the environmental chambers determined phenol as the largest phenolic compound based on a mass basis, but it should be noted that phenol was also measured at elevated levels in the inlet air duct. Field blank samples had no quantifiable levels of any of the target compounds.

The **alcohols** including ethanol (EtOH) and methanol (MeOH) were emitted at high fluxes during all periods in which waste was present in the chamber. Enteric fermentation contributed to alcohol emissions but fresh waste was clearly the main contributor.
All **amine** fluxes were below the LOQ in all experimental periods.

Table 6: Methane emissions (lbs/cow/yr) from dry cows and waste and lactating cows and waste during Exp. 1 & 2 (concentrations were measured using INNOVA analyzers).
Note: The "Cows only" category is similar to "Cows & waste" indicating that enteric fermentation is a major contributor to total emissions.

	Avg Emissions (lb/cow/yr)			
	Methane			
	Empty	**Cows only**	**Cows & Waste**	**Waste only**
Dry Cows, exp.1	0.57	334.77	236.48	2.46
Lact. Cows, exp.1	0.49	340.21	350.88	2.05
Dry Cows, exp.2	2.49	215.17	222.48	N/A
Lact. Cows, exp.2	N/A	388.55	354.06	7.53
	Emissions (lb/cow/yr) per Group			
	Methane			
Exp.1	**Empty**	**Cows only**	**Cows & Waste**	**Waste only**
Dry #1	N/A	N/A	N/A	N/A
Dry #2	N/A	N/A	N/A	N/A
Dry #3	0.57	334.77	236.48	2.46
Average	**0.57**	**334.77**	**236.48**	**2.46**
Standard Deviation	N/A	N/A	N/A	N/A
Exp.1				
Lact #1	0.75	345.83	363.36	3.43
Lact #2	0.41	407.69	399.92	0.68
Lact #3	0.29	257.60	309.26	1.97
Lact #4	0.52	349.73	330.98	2.14
Average	**0.49**	**340.21**	**350.88**	**2.05**
Standard Deviation	0.19	61.91	39.53	1.13
Exp.2				
Dry #4	2.49	121.10	194.83	N/A
Dry #5	N/A	252.61	225.60	N/A
Dry #6	N/A	215.70	234.01	N/A
Dry #7	N/A	271.27	235.47	N/A
Average	**2.49**	**215.17**	**222.48**	**N/A**
Standard Deviation	N/A	66.83	18.94	N/A
Exp. 2				
Lact. 5	N/A	422.22	351.72	N/A
Lact. 6	N/A	354.87	356.39	7.53
Average	**N/A**	**388.55**	**354.06**	**7.53**
Standard Deviation	N/A	47.62	3.30	N/A

Table 7: Volatile fatty acid averages (lbs/cow/yr) and standard deviation emissions from dry cows and waste, and lactating cows and waste during Exp. 1.

Period	*Treatment*	*Acetic Acid*	*Propionic Acid*	*Isobutyric Acid*	*Butyric Acid*	*Isovaleric Acid*	*Valeric Acid*
1st Dry	Empty	0.074 ± N/A	0	0	0.245 ± N/A	0	0.016 ± N/A
1st Dry	Cows only	0.175 ± N/A	0	0	0.240 ± N/A	0	0.020 ± N/A
1st Dry	Cows & Waste	0.031 ± 0.033	0.016 ± 0.025	0	0.068 ± 0.072	0	0.009 ± 0.010
1st Dry	Waste only	0.009 ± 0.017	0.011 ± 0.015	0	0.123 ± 0.189	0	0.005 ± 0.009
2nd Dry	Empty	0.012 ± N/A	0	0	0	0	0
2nd Dry	Cows only	0.017 ± N/A	0	0	0	0	0
2nd Dry	Cows & Waste	0.003 ± 0.006	0	0	0.009 ± 0.019	0	0
2nd Dry	Waste only	0	0	0	0	0	0
3rd Dry	Empty	0	0	0	0	0	0
3rd Dry	Cows only	0.013 ± N/A	0	0	0	0	0
3rd Dry	Cows & Waste	0.023 ± 0.016	0.007 ± 0.014	0	0.009 ± 0.019	0	0
3rd Dry	Waste only	0.010 ± 0.008	0	0	0	0	0
1st Lact.	Empty	0	0	0	0	0	0
1st Lact.	Cows only	0	0	0	0	0	0
1st Lact.	Cows & Waste	0.022 ± 0.006	0	0	0	0	0
1st Lact.	Waste only	0.003 ± 0.006	0	0	0	0	0
2nd Lact.	Empty	0	0	0	0	0	0
2nd Lact.	Cows only	0.016 ± N/A	0	0	0	0	0
2nd Lact.	Cows & Waste	0.043 ± 0.024	0.048 ± 0.067	0	0.040 ± 0.001	0	0
2nd Lact.	Waste only	0.051 ± 0.034	0.043 ± 0.036	0	0.038 ± 0.029	0	0
3rd Lact.	Empty	0	0	0	0	0	0
3rd Lact	Cows only	0.028 ± N/A	0	0	0	0	0
3rd Lact.	Cows & Waste	0.027 ± 0.009	0	0	0.037 ± 0.002	0	0
3rd Lact	Waste only	0.004 ± 0.006	0	0	0	0	0

Table 8: Volatile fatty acid and phenolic compound averages and standard deviation emissions from dry cows and waste and lactating cows and waste during Exp. 1.

Period	*TREATMENT*	*Isocaproic Acid*	*Caproic Acid*	*Heptanoic Acid*	*2-methyl phenol*	*phenol*	*2-ethyl phenol*	*3/4-methyl phenol*
1st Dry	Empty	0	0	0	0.006 ± N/A	0.047 ± N/A	0	0.008 ± N/A
1st Dry	Cows only	0	0	0	0.008 ± N/A	0.039 ± N/A	0.009 ± N/A	0.023 ± N/A
1st Dry	Cows & Waste	0	0	0	0.005 ± 0.000	0.016 ± 0.019	0.016 ± 0.021	0.022 ± 0.011
1st Dry	Waste only	0	0	0	0.007 ± 0.006	0.015 ± 0.017	0.086 ± 0.063	0.076 ± 0.065
2nd Dry	Empty	0	0	0	0.005 ± N/A	0.007 ± N/A	0.003 ± N/A	0.036 ± N/A
2nd Dry	Cows only	0	0	0	0.004 ± N/A	0	0.023 ± N/A	0.035 ± N/A
2nd Dry	Cows & Waste	0	0	0	0.002 ± 0.002	0.007 ± 0.014	0.022 ± 0.016	0.047 ± 0.062
2nd Dry	Waste only	0	0	0	0.004 ± 0.001	0.004 ± 0.007	0.034 ± 0.032	0.046 ± 0.035
3rd Dry	Empty	0	0	0	0.004 ± N/A	0	0	0.013 ± N/A
3rd Dry	Cows only	0	0	0	0.003 ± N/A	0.011 ± N/A	0.016 ± N/A	0.055 ± N/A
3rd Dry	Cows & Waste	0	0	0	0.006 ± 0.002	0.037 ± 0.026	0.034 ± 0.023	0.053 ± 0.015
3rd Dry	Waste only	0	0	0	0.005 ± 0.001	0.047 ± 0.019	0.095 ± 0.079	0.094 ± 0.030
1st Lact.	Empty	0	0	0	0.004 ± N/A	0.017 ± N/A	0.034 ± N/A	0
1st Lact.	Cows only	0	0	0	0.004 ± N/A	0.006 ± N/A	0.014 ± N/A	0
1st Lact.	Cows & Waste	0	0	0	0.008 ± 0.000	0.044 ± 0.004	0.132 ± 0.062	0.099 ± 0.006
1st Lact.	Waste only	0	0	0	0.005 ± 0.001	0.019 ± 0.005	0.058 ± 0.045	0.074 ± 0.027
2nd Lact.	Empty	0	0	0	0.010 ± N/A	0.016 ± N/A	0.049 ± N/A	0.039 ± N/A
2nd Lact.	Cows only	0	0	0	0.011 ± N/A	0.011 ± N/A	0.036 ± N/A	0.036 ± N/A
2nd Lact.	Cows & Waste	0	0	0	0.021 ± 0.001	0.033 ± 0.001	0.035 ± 0.049	0.091 ± 0.020
2nd Lact.	Waste only	0	0	0	0.010 ± 0.005	0.026 ± 0.014	0.079 ± 0.073	0.105 ± 0.072
3rd Lact.	Empty	0	0	0	0.014 ± N/A	0.005 ± N/A	0.049 ± N/A	0.043 ± N/A
3rd Lact	Cows only	0	0	0	0.018 ± N/A	0.025 ± N/A	0	0.071 ± N/A
3rd Lact.	Cows & Waste	0	0	0	0.020 ± 0.000	0.031 ± 0.007	0.114 ± 0.019	0.090 ± 0.024
3rd Lact	Waste only	0	0	0	0.018 ± 0.005	0.024 ± 0.011	0.049 ± 0.034	0.077 ± 0.035

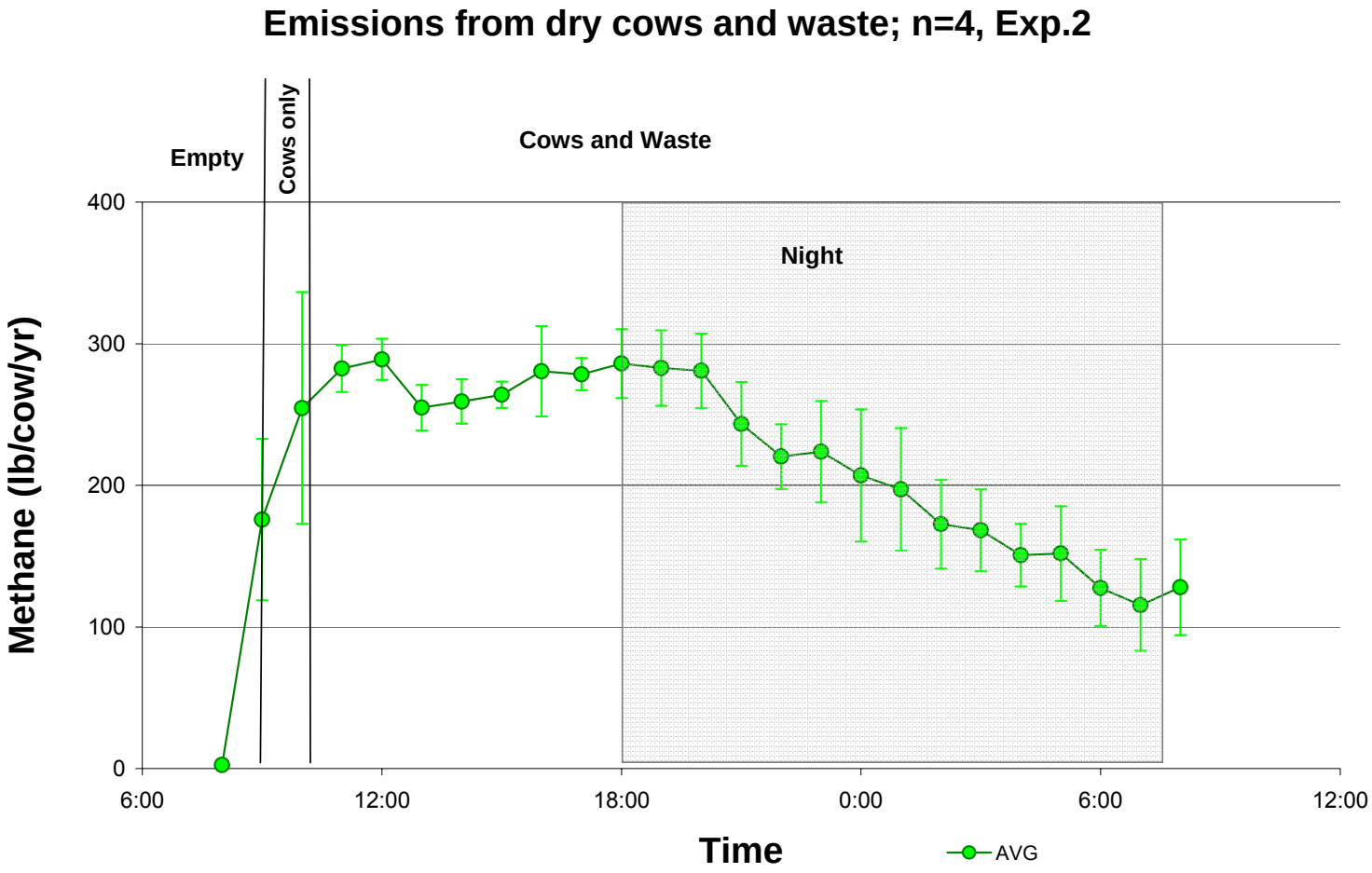

Figure 1: Average methane emissions (error bars indicate standard deviation) from four groups of dry cows and waste during Exp. 2. Note: Upon entry of cows into chambers, methane flux immediately increased indicating that enteric fermentation is the main process in the formation of this gas. After removal of cows from chambers, methane went back to background concentrations.

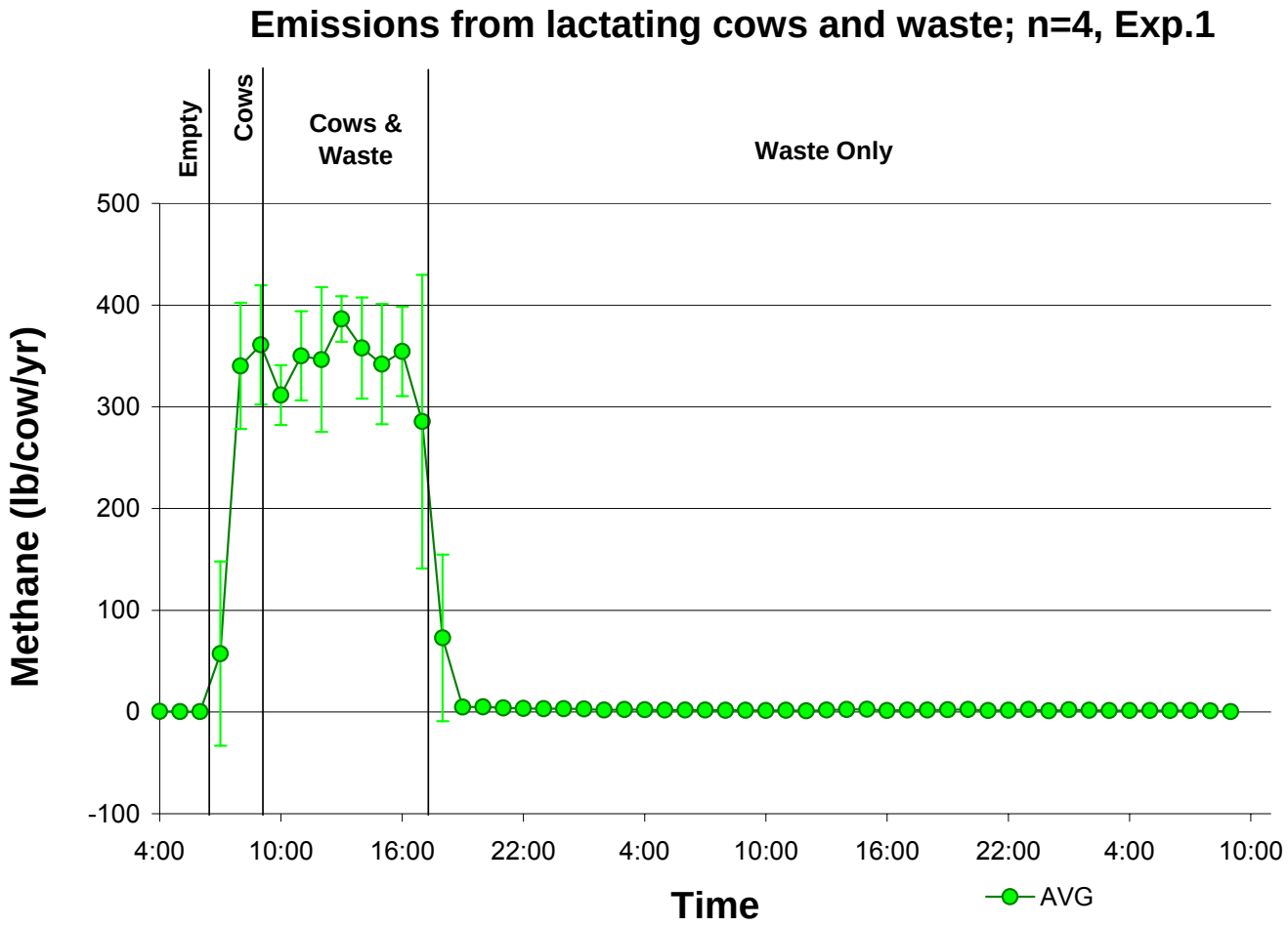

Figure 2: Average methane emissions (error bars indicate standard deviation) from four groups of lactating cows and waste during Exp. 1. Note: Upon entry of cows into chambers, methane flux immediately increased indicating that enteric fermentation is the main process in methane formation. Soon after removal of cows, methane flux returned to background levels.

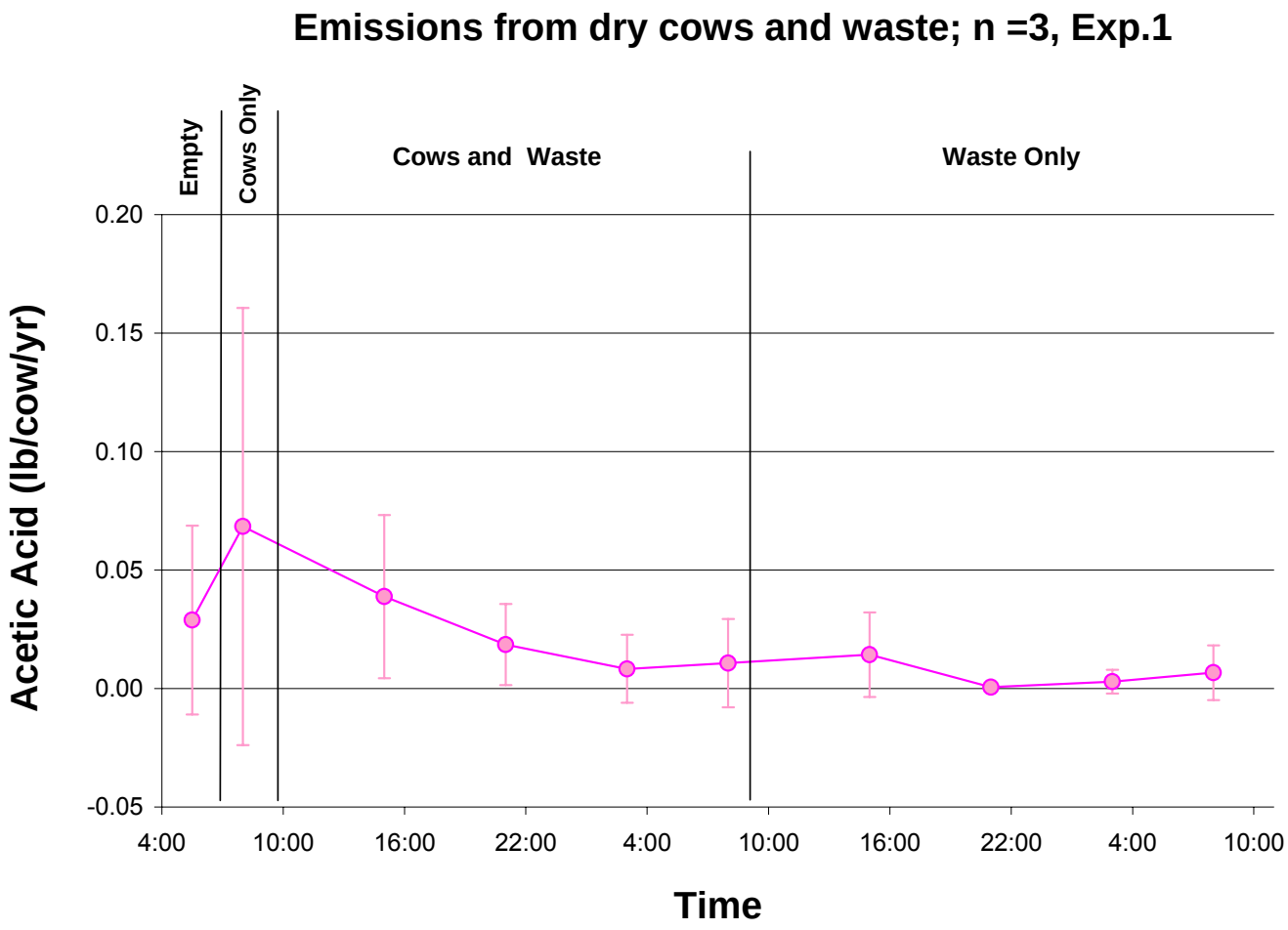

Figure 3: Average acetic acid emissions (error bars indicate standard deviation) from three groups of dry cows and waste during Exp. 1. Note: Upon entry of cows into chambers, acetic acid flux increased indicating that enteric fermentation is a responsible process in the formation of this gas. Acetic acid decreased over time indicating that fresh waste is a minor factor in its production. Concentrations near the detection limit of the assay, make further interpretation of tends difficult.

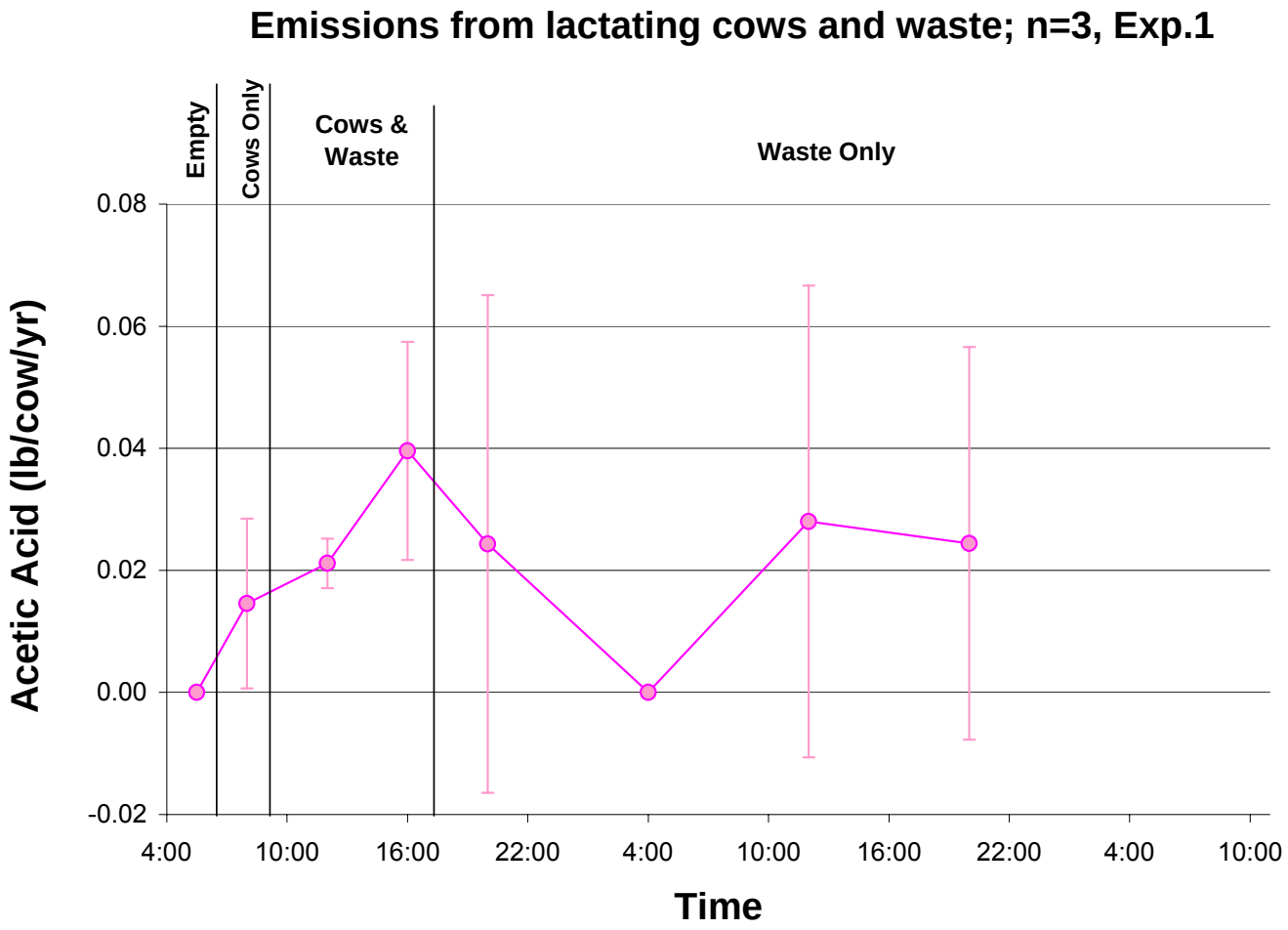

Figure 4: Average acetic acid emissions (error bars indicate standard deviation) from three groups of lactating cows and waste during Exp. 1. Note: Upon entry of cows into chambers, acetic acid flux increased indicating that enteric fermentation is a responsible process in the formation of this gas. High variability across the three cow groups and concentrations near the lower detection limit of the assay, makes further interpretation of tends difficult.

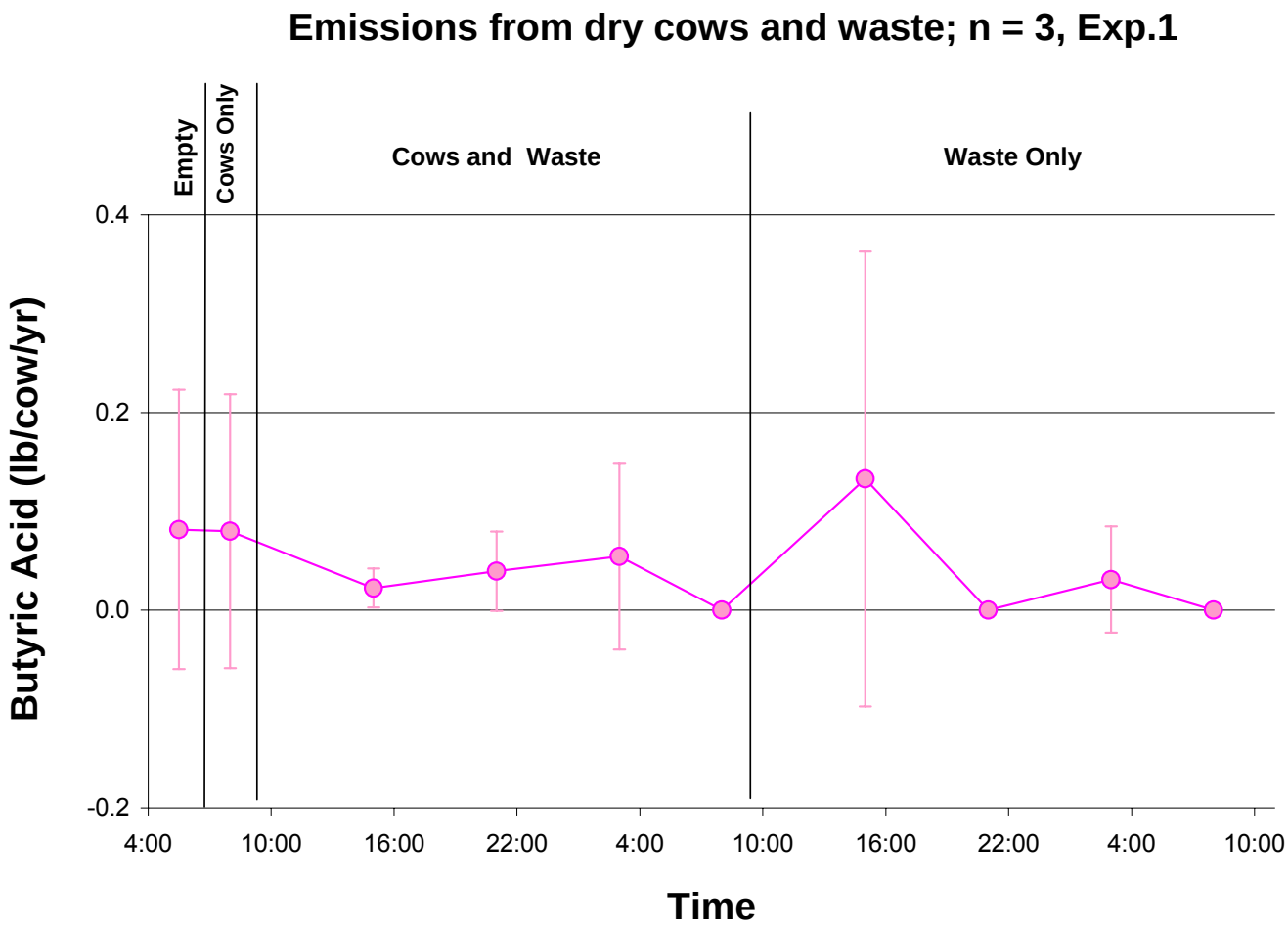

Figure 5: Average butyric acid emissions (error bars indicate standard deviation) from three groups of dry cows and waste during Exp. 1. Note: Butyric acid fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

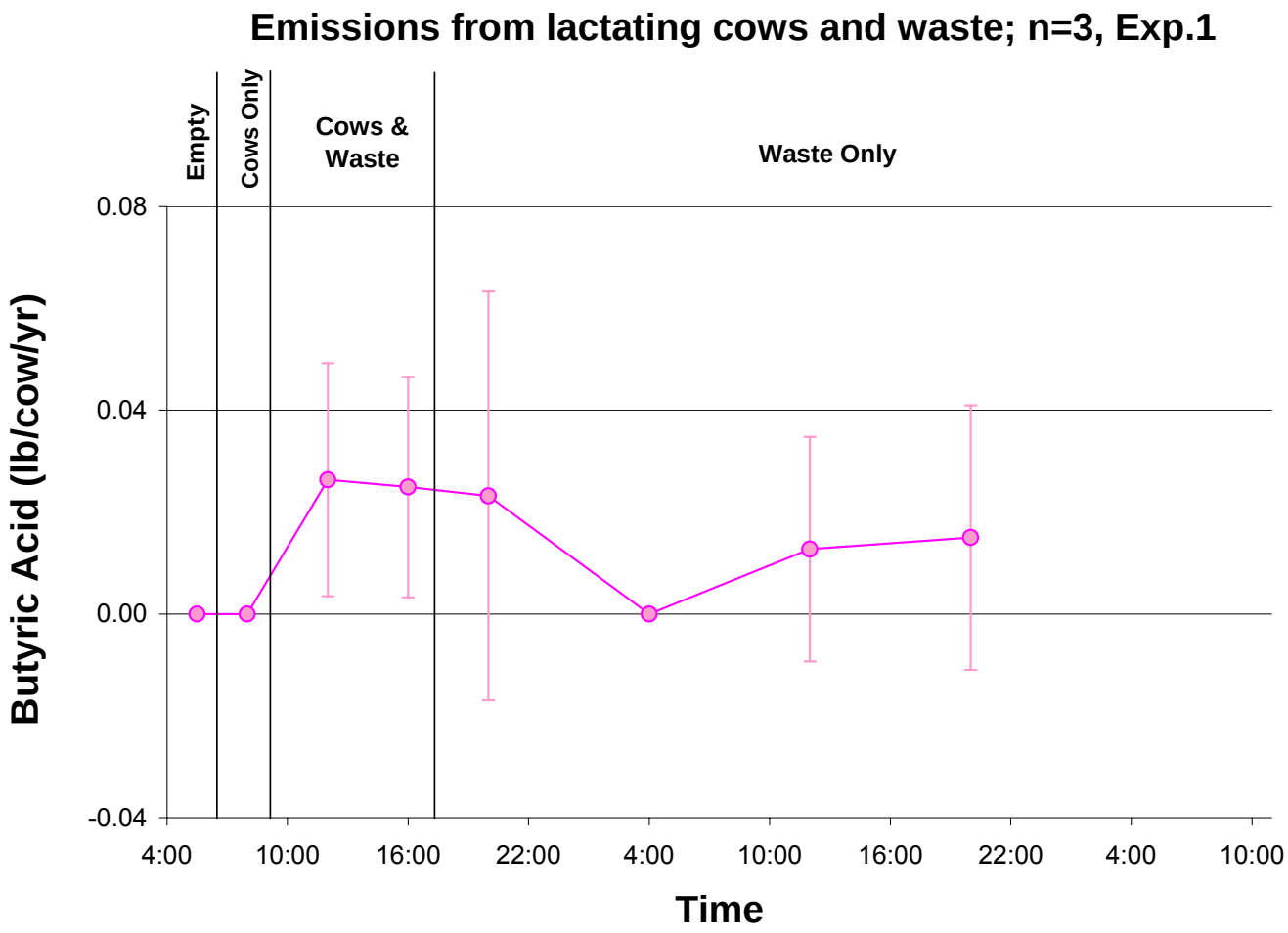

Figure 6: Average butyric acid emissions (error bars indicate standard deviation) from three groups of lactating cows and waste during Exp. 1. Note: Butyric acid fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

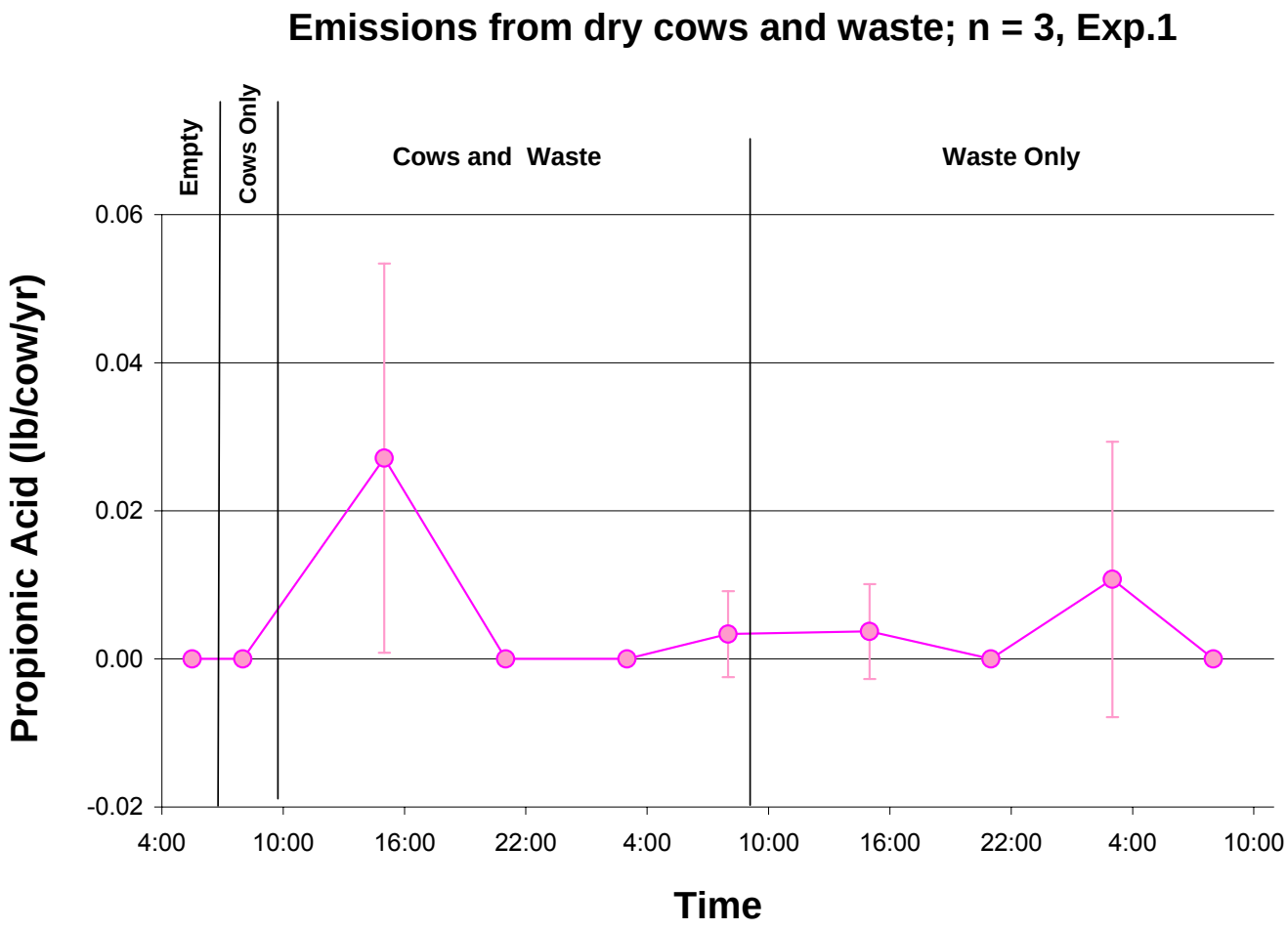

Figure 7: Average propionic acid emissions (error bars indicate standard deviation) from three groups of dry cows and waste during Exp. 1. Note: Propionic acid fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

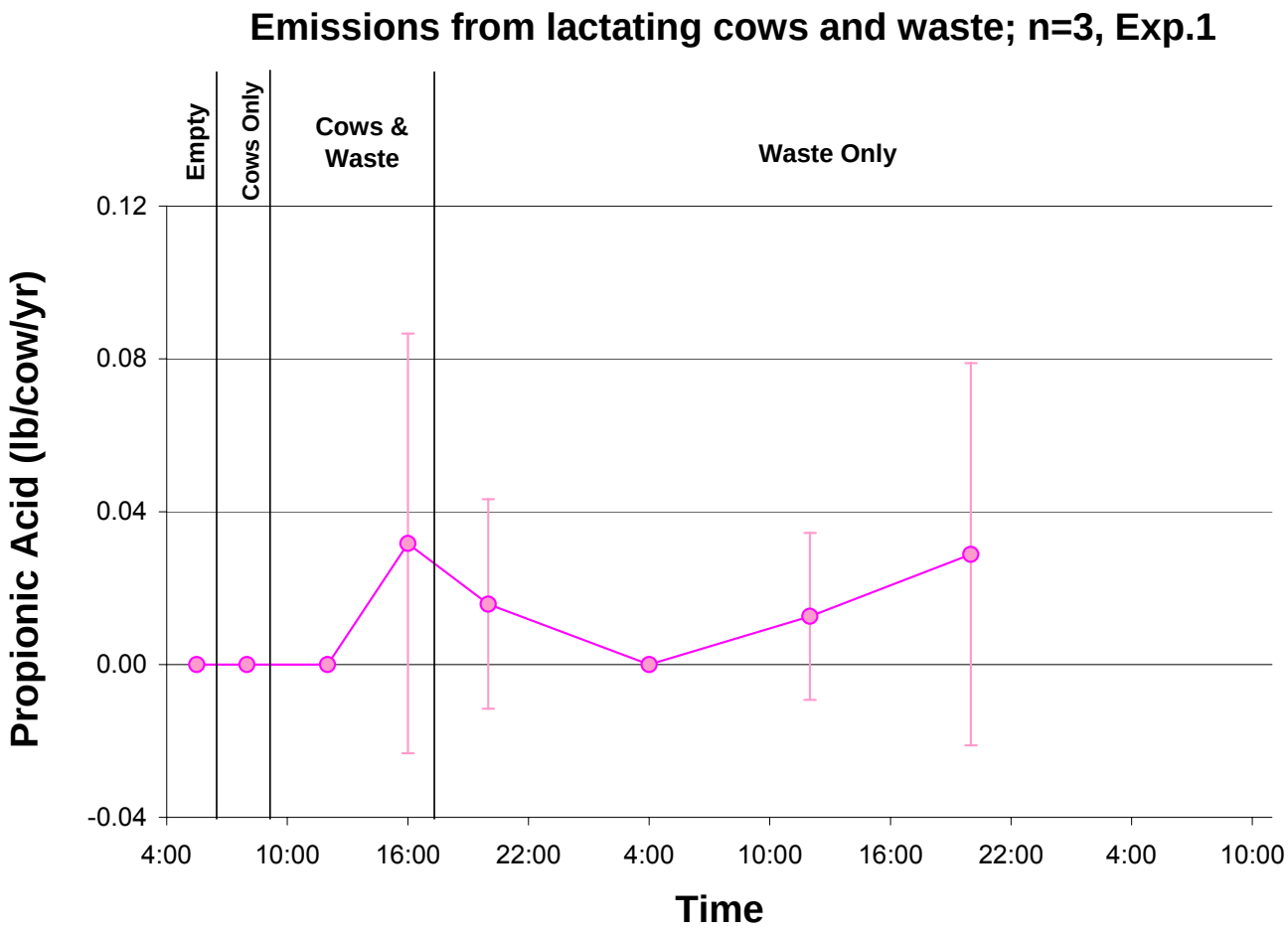

Figure 8: Average propionic acid emissions (error bars indicate standard deviation) from three groups of lactating cows and waste during Exp. 1. Note: Propionic acid fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

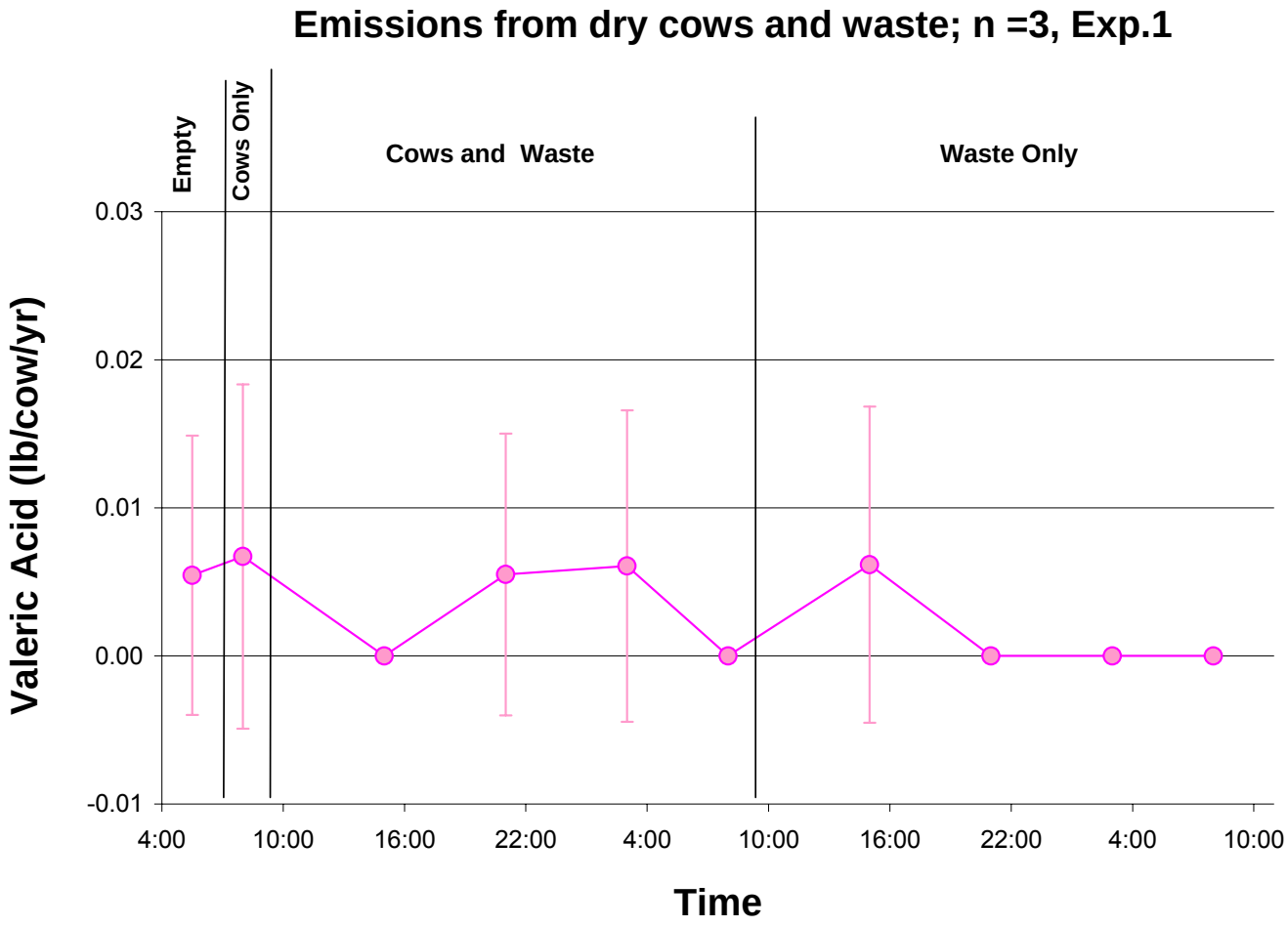

Figure 9: Average valeric acid emissions (error bars indicate standard deviation) from three groups of dry cows and waste during Exp. 1. Note: Valeric acid fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

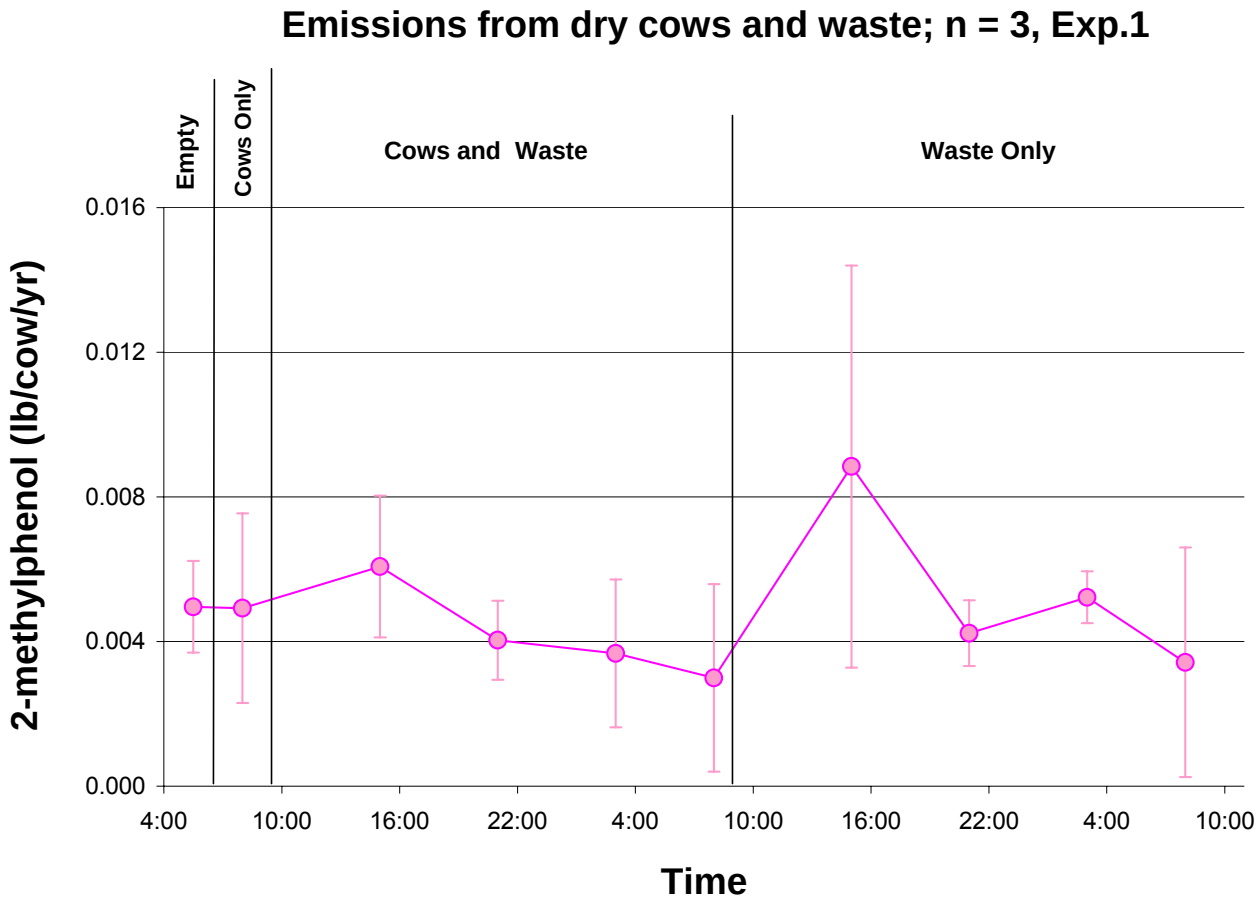

Figure 10: Average 2-methylphenol emissions (error bars indicate standard deviation) from three groups of dry cows and waste during Exp. 1. Note: 2-methylphenol fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

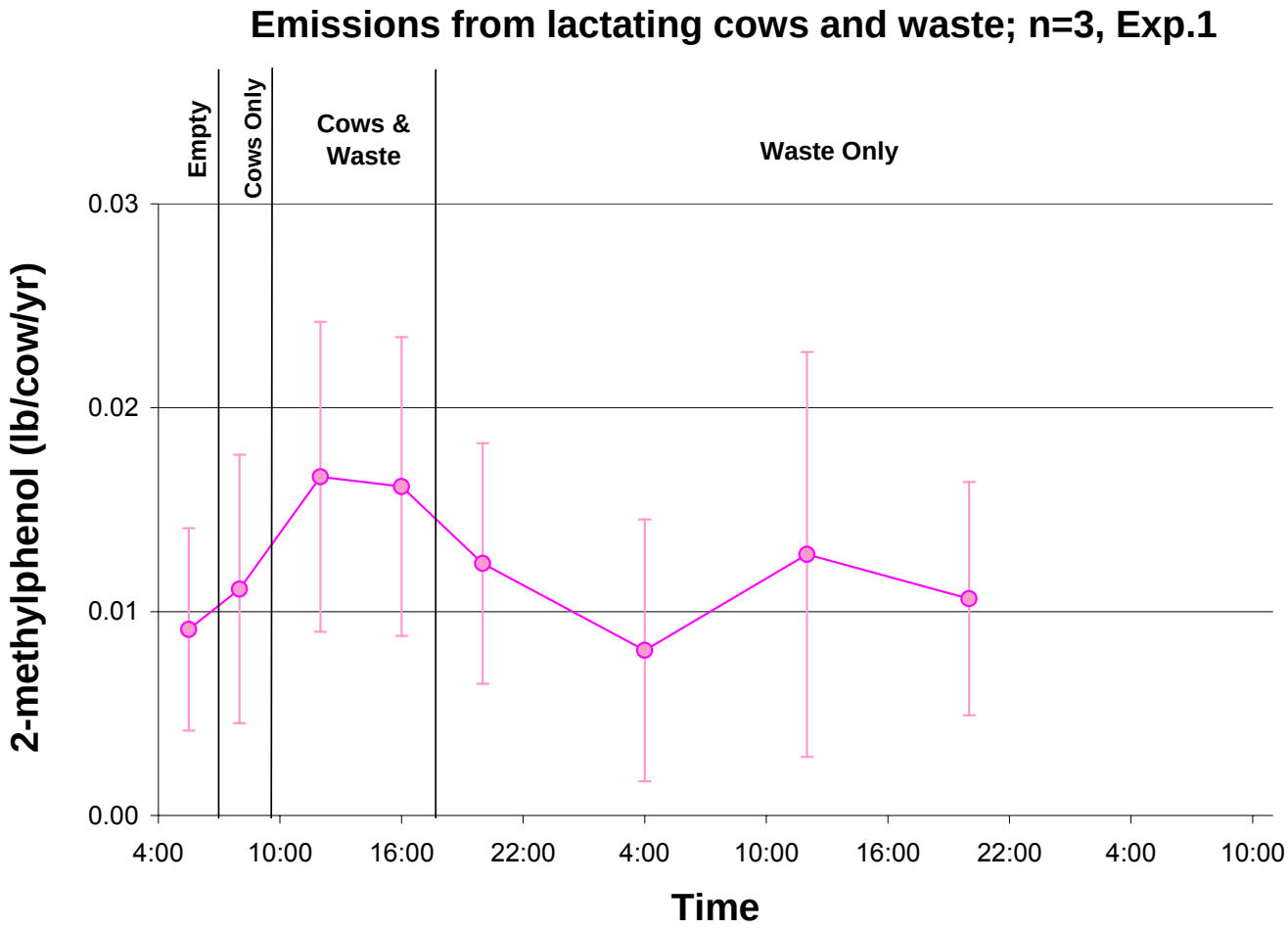

Figure 11: Average 2-methylphenol emissions (error bars indicate standard deviation) from three groups of lactating cows and waste during Exp. 1. Note: 2-methylphenol fluxes across cow groups were variable and the concentrations were near the lower detection limit of the assay.

Table 9: Methanol and ethanol emissions from four groups of dry cows and waste during Exp. 2. During the dry cow iterations in Exp. 2, "Waste only" was not measured.

	Average Dry Cows Emissions (lb/cow/yr)							
	Methanol				**Ethanol**			
	Empty	**Cows only**	**Cows & Waste**	**Waste only**	**Empty**	**Cows only**	**Cows & Waste**	**Waste only**
Exp.2								
Dry # 4	0.23	0.63	2.50	N/A	0.43	0.30	2.67	N/A
Dry # 5	N/A	3.10	2.80	N/A	N/A	1.18	2.67	N/A
Dry # 6	N/A	2.10	3.49	N/A	N/A	0.70	3.57	N/A
Dry # 7	N/A	1.98	3.57	N/A	N/A	2.09	4.90	N/A
Average	**N/A**	**1.95**	**3.09**	**N/A**	**N/A**	**1.07**	**3.45**	**N/A**
Standard Deviation	**N/A**	**1.01**	**0.52**	**N/A**	**N/A**	**0.77**	**1.06**	**N/A**

Table 10: Methanol and ethanol emissions from four groups of lactating cows and waste during Exp. 2.
Note: The "Cows only" category is considerably lower compared to "Cows & waste" indicating that waste is a major contributor to total emissions. During this experiment, "Waste only" was measured only for lactating cow group # 6.
Lactating cows in every Exp 2 group were housed inside the chamber for 24 hrs and milked at 8 a.m. and 7 p.m.

Average Lactating Cow Emissions (lb/cow/yr)

	Methanol				**Ethanol**			
	Empty	**Cows only**	**Cows & Waste**	**Waste only**	**Empty**	**Cows only**	**Cows & Waste**	**Waste only**
Exp. 2								
Lact. # 5	N/A	2.37	11.41	N/A	N/A	2.06	15.81	N/A
Lact. # 6	N/A	4.31	10.82	8.57	N/A	2.25	14.16	14.56
Lact. # 7	0.13	0.39	N/A	N/A	0.02	0.31	N/A	N/A
Average	**N/A**	**3.34**	**11.12**	**N/A**	**N/A**	**2.16**	**14.98**	**N/A**
Standard Deviation	**N/A**	**1.37**	**0.42**	**N/A**	**N/A**	**0.13**	**1.17**	**N/A**

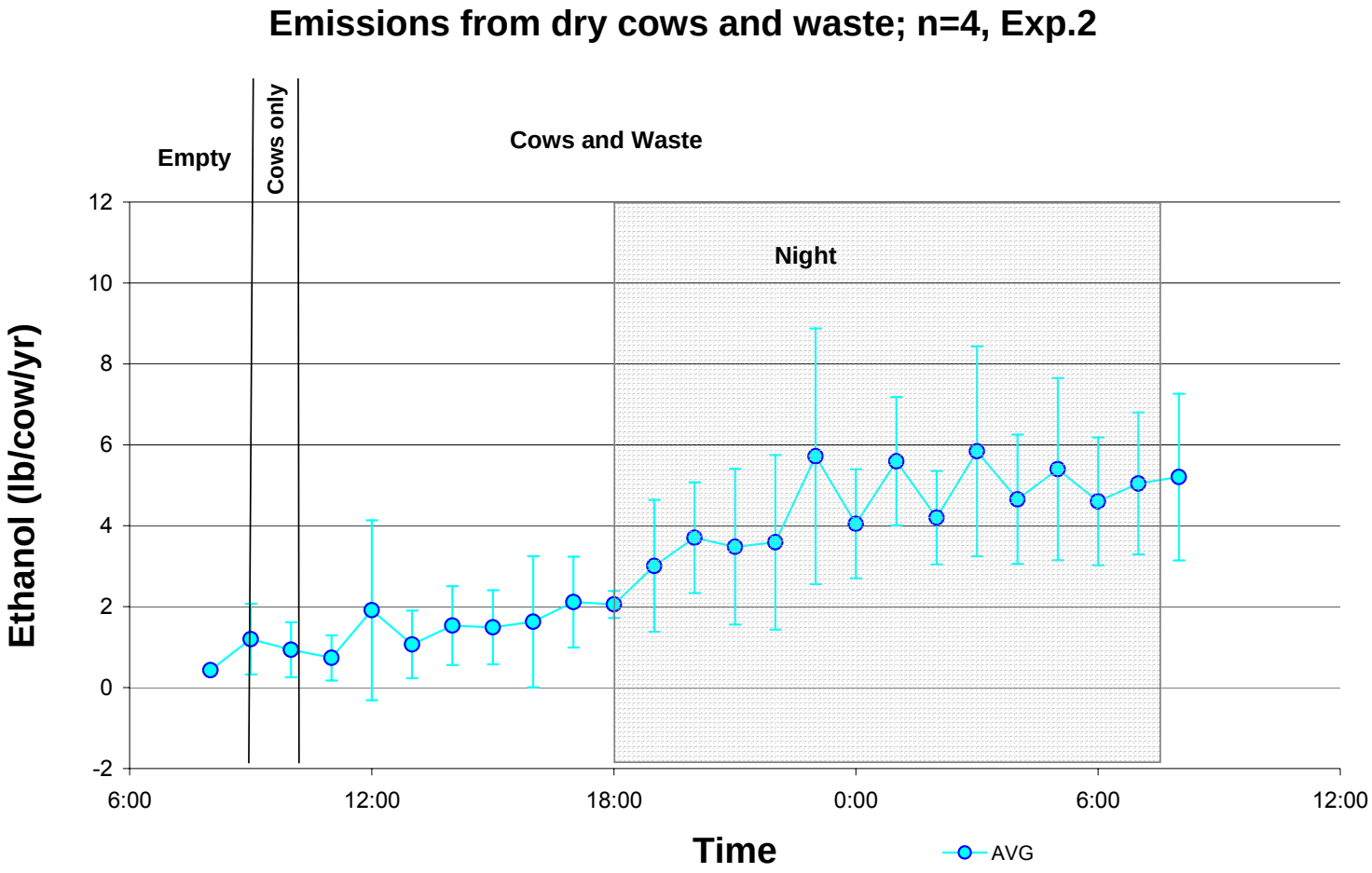

Figure 12: Average ethanol emissions (error bars indicate standard deviation) from four groups of dry cows and waste during Exp. 2. Note: Upon entry of cows into chambers, ethanol flux only slightly increased indicating that enteric fermentation is a minor process in the formation of this gas. Ethanol increased over time with increasing accumulation of waste.

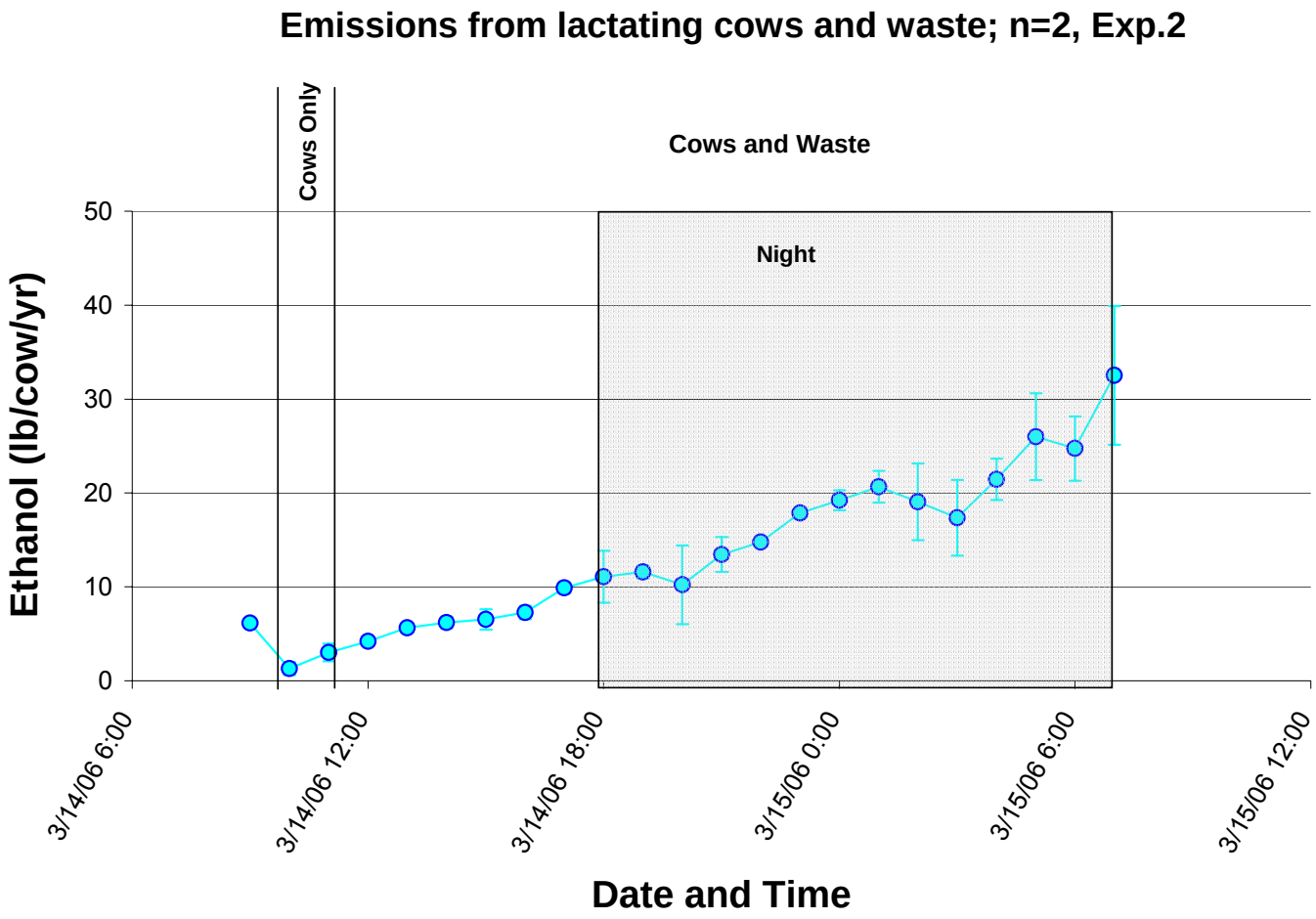

Figure 13: Average ethanol emissions (error bars indicate standard deviation) from two groups or lactating cows and waste during Exp. 2. Note: Upon entry of cows into chambers, ethanol flux was minimal indicating that enteric fermentation is a minor process in the formation of this gas. Ethanol increased over time with increasing accumulation of waste and reached a very high flux after 24 hrs. Under the present conditions, waste was not flushed or scraped but remained in the chamber.

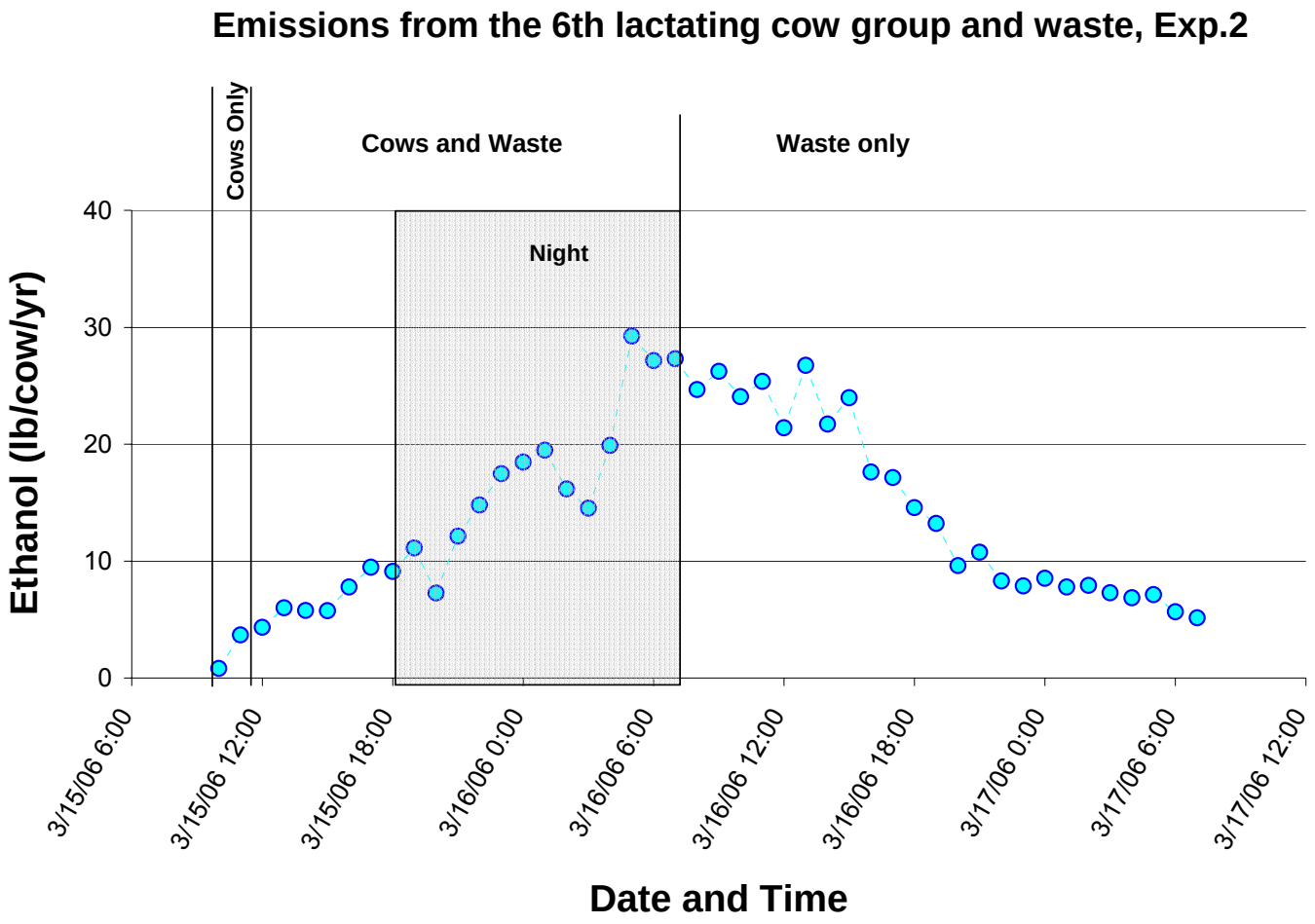

Figure 14: Ethanol emissions from lactating cow group #6 and waste during Exp. 2 (no flushing). Note: Upon entry of cows into chambers, ethanol flux increase moderately. Ethanol increased over time coinciding with increasing accumulation of waste and reaches a high flux after 24 hrs. In the "waste only" phase, EtOH remained high for several hours indicating that indeed waste is the main source. The following decrease within the "waste only" phase might be related to a decrease in fermentable cellulose and microorganism activities in the feces.

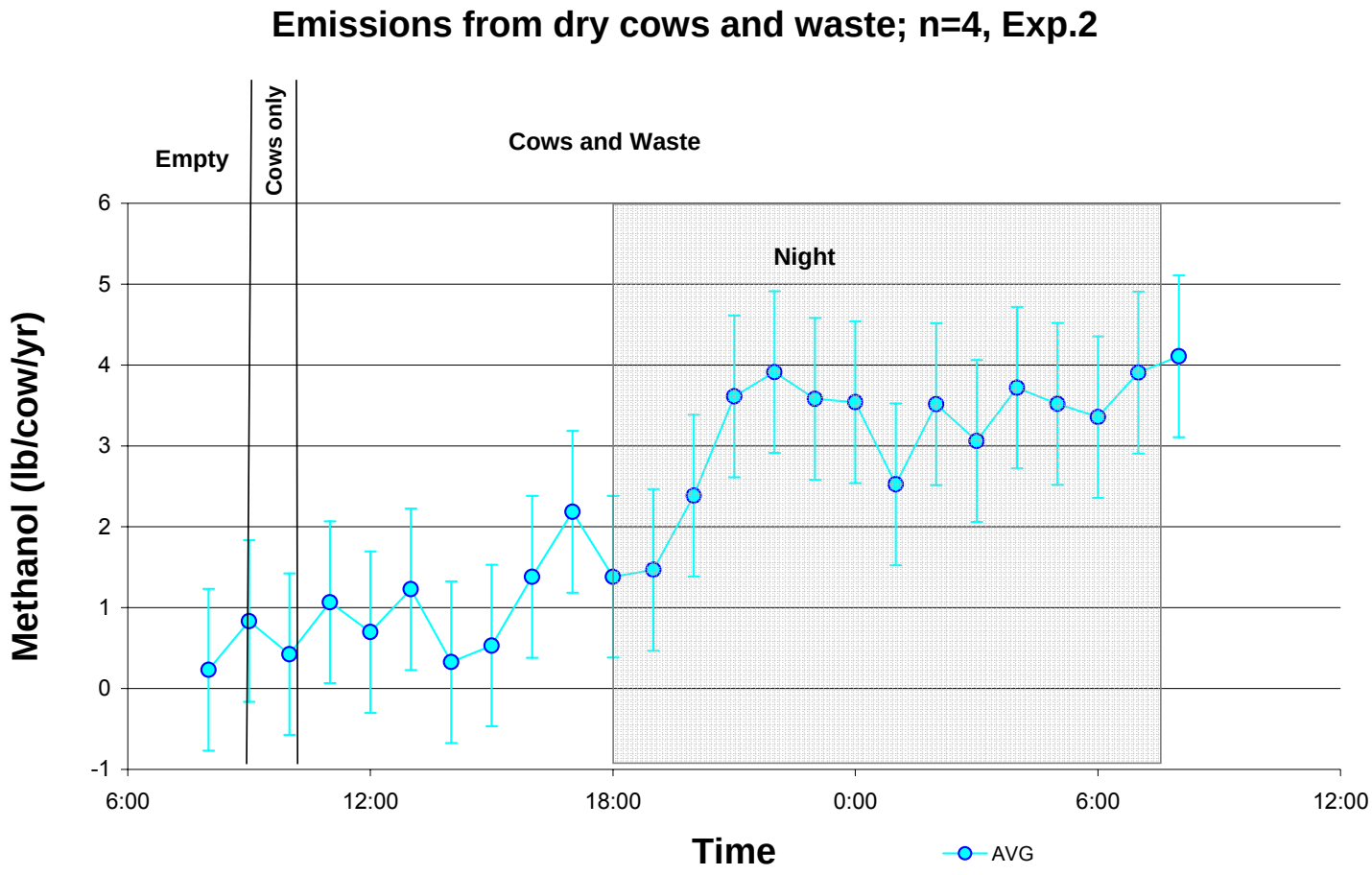

Figure 15: Average methanol emissions (error bars indicate standard deviation) from four groups of dry cows and waste during Exp. 2. Note: Upon entry of cows into chambers, methanol flux only slightly increased indicating that enteric fermentation is a minor process in the formation of this gas. Methanol increased over time with increasing accumulation of waste.

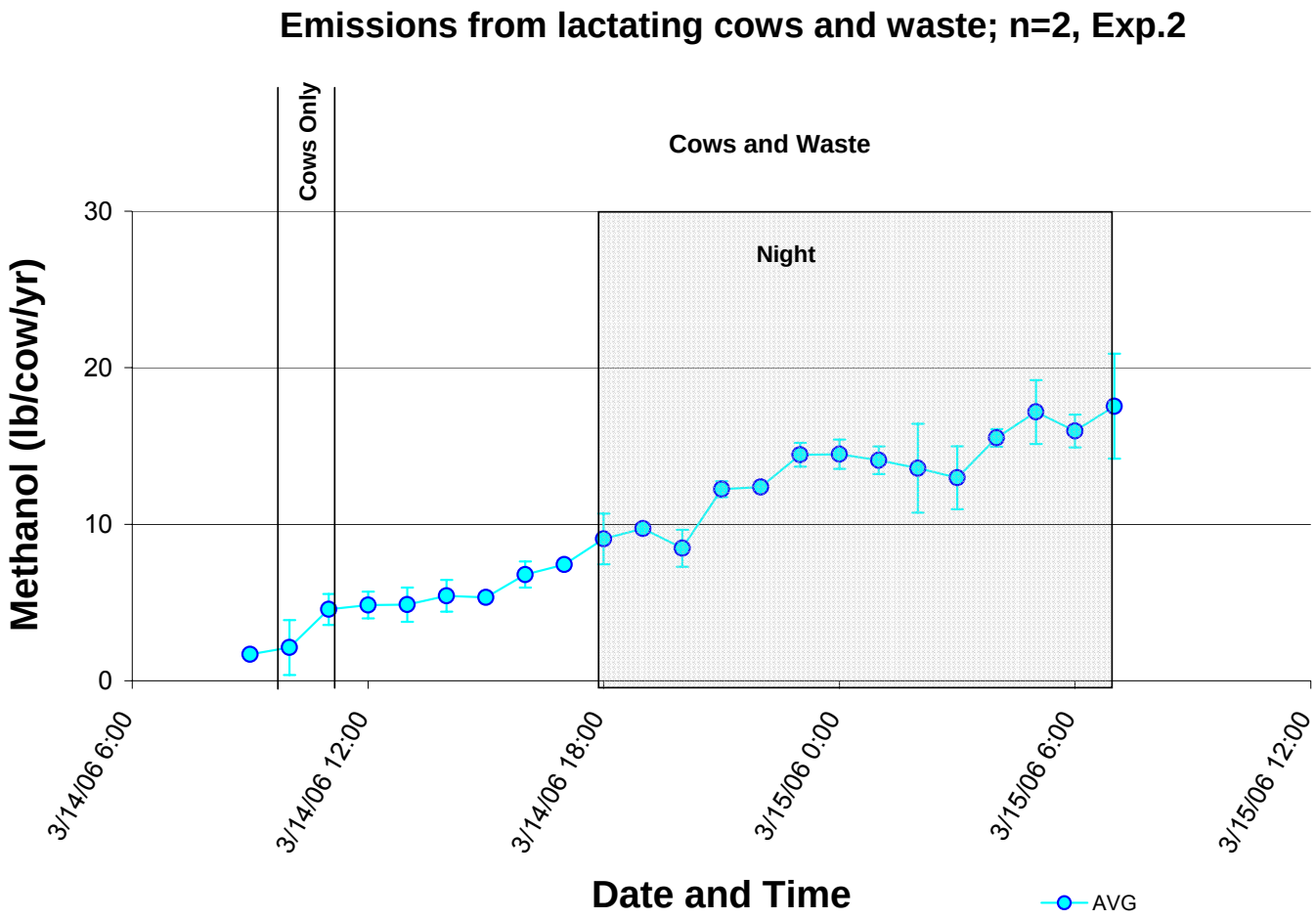

Figure 16: Average methanol emissions (error bars indicate standard deviation) from two groups of lactating cows and waste during Exp. 2. Note: Upon entry of cows into chambers, methanol flux increased moderately. Methanol increased over time with increasing accumulation of waste and reached a very high flux after 24 hrs. Under the present conditions, waste was not flushed or scraped but remained in the chamber.

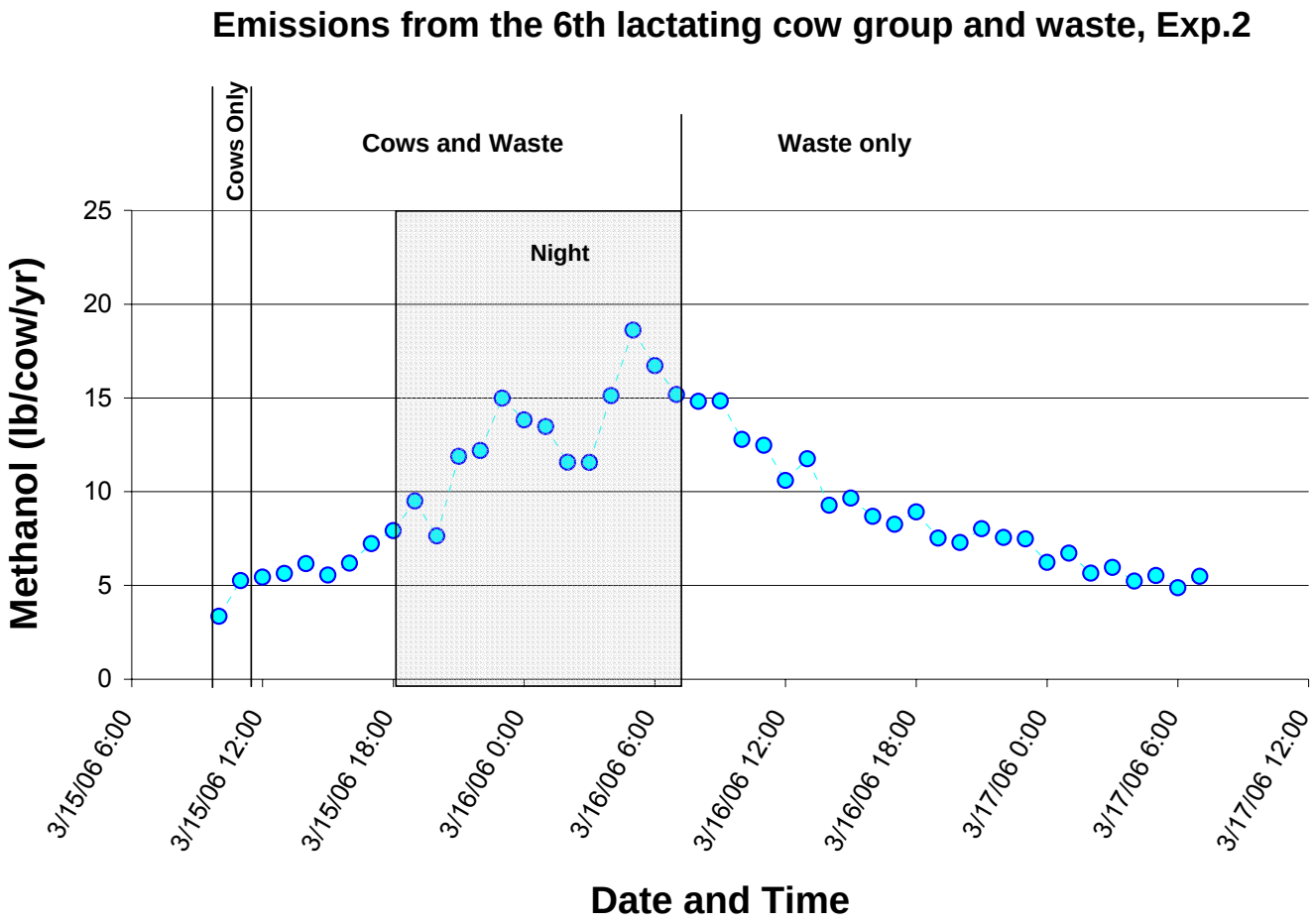

Figure 17: Methanol emission factors from the 6th lactating cow group and waste during Exp. 2 (no flushing).
Note: Upon entry of cows into chambers, methanol flux was moderate. Methanol increased over time with increasing accumulation of waste and reaches a very high flux after 24 hrs. In the "waste only" phase, MeOH remained high for several hours but showed a decreasing trend. The decreasing trend within the "waste only" phase might be related to a decrease in fermentable cellulose and microorganism activities in the feces. Under the present conditions, waste was not flushed or scraped but remained in the chamber.

6 DISCUSSION

Methane is produced during enteric fermentation in the cow's rumen. It is generally estimated that 6% of the energy consumed by cows is eructated in form of methane. Our experiments showed immediate increases of methane fluxes as soon as cows were introduced into the chamber. Differences between dry and lactating cows were expected and observed. Lactating cows produced approximately 1/3 more methane than non-lactating dry cows. Fresh waste did not produce noticeable methane fluxes.

Currently, there are no other VFA studies to directly compare to the present study; however, two studies measuring the air quality within dairy barns have been conducted. In these studies, the total number of cows per barn varied from 25-169 (Martensson et al., 1999; Sonesson et al., 2001). Martensson et al. (1999) monitored only VFA compounds and found that acetic acid concentrations in air ranged from 31-78 μg m^{-3}, while butyric acid concentration ranged from 4-11 μg m^{-3}. If we scaled our numbers to reflect similar population sizes in dairy cows, our acetic acid concentration would have ranged from 36-247 μg m^{-3} and our butyric acid concentrations would have ranged from 0-64 μg m^{-3}. In addition, Sonesson et al. (2001) reported that only isovaleric acid was detected at 0.1-0.8 μg m^{-3}, concentrations which are lower than our method LOQ of 3.7 mg m^{-3}. Sonesson et al. (2001) also reported detection of phenol (3-50 μg m^{-3}), 4-methylphenol (0.6-100 μg m^{-3}), and 4-ethylphenol (0.4-10 μg m^{-3}). If we scaled our numbers to reflect similar population size in dairy cows, our phenol concentration would have ranged from 9.6-50.7 μg m^{-3} and our 4-methylphenol concentrations would have ranged from 21.9-200 μg m^{-3}. VFAs and phenolic compounds reported in the present study are similar in concentration magnitudes as those reported previously.

Both methanol and ethanol emission fluxes from dry and lactating cows and waste were high. Upon entry of cows into chambers, ethanol and methanol fluxes increased only slightly indicating that enteric fermentation was a minor process in the formation of these gases. Both alcohols increased over time coinciding with increasing accumulation of waste and reached the highest flux after 24 hrs. In the "waste only" phase without cows present, both alcohols remained high for several hours indicating that indeed waste is the main alcohol source. The following decrease within the "waste only" phase might be related to a decrease in fermentable sugars and cellulose in the feces (see also Figure 18).

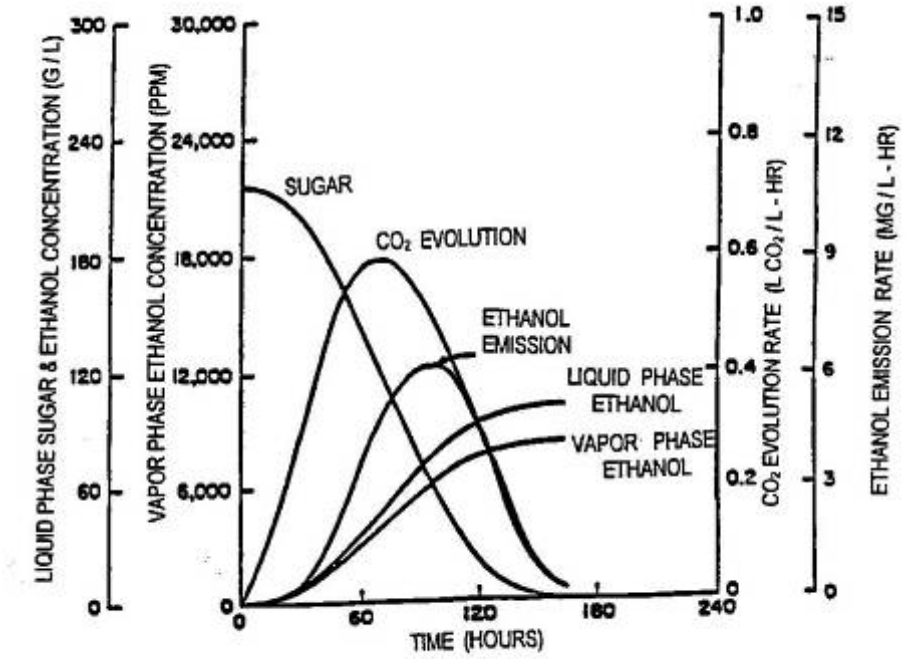

Figure: 18: Fermentation relationship between sugar consumption and ethanol production. Ethanol is produced until a critical mass of fermentable sugars is present. Once the majority of sugars are consumed by microbes, ethanol production decreases (Williams, 1983).

The conceptual relationship shown in Figure 18 was observed in the present study. Both ethanol and methanol increased with increasing amounts of manure (and therefore cellulose and sugar) in the chamber. During the first few hours in the “waste only” phase alcohols remained high but decreased over time, most likely due to decrease in the availability of cellulose and sugars to bacteria and the fact that the conditions to anaerobic bacteria in fresh waste are suboptimal and detrimental.

FUTURE STUDIES

The present study showed large differences in emissions between dry and lactating cows due to the dietary rations and physiological stages (dry/lactating). Future studies which focus on the impacts of nutrition on emission profiles are needed.

In addition, we have conducted a preliminary study to test the effects of flushing dairy waste. The results indicated that flushing of manure wastes from the chambers floor reduced alcohol emissions. Because alcohols are highly soluble in water, it is possible that the addition of flush water could keep the alcohol in solution where it is broken down into other compounds. Further study of the dairy industry's standard practice of flushing freestalls will be useful to evaluate its impact on alcohol emission mitigation, and the ultimate fate of alcohols in the waste stream.

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8 APPENDIX

DETAILED MATERIALS AND METHODS

VFAs and PHENOLS

Method Validation

Prior to the initiation of the study the following method validation procedures were performed: 1) evaluate stability of target compounds during analysis; 2) determine safe sampling volume (SSV); and 3) verify storage stability. Stability of target analytes was tested by loading a known quantity of reference standard onto five sorbent tubes and loading five empty glass tubes with same quantity of reference standard. Area counts for each target compound were determined and compared for both sorbent tubes and empty tubes. No significant difference in the area counts was noted so transformation of target compounds during analysis was considered low to negligible (data not shown). The SSV for the sorbent tubes was tested by loading sorbent tubes with reference standards and challenged sorbent tubes with 2, 4, 6 and 12 L of air (nitrogen). The SSV was confirmed by challenging sorbent tubes with 12 L of air at 100 mL min^{-1} under ambient temperatures (24^{o}C) and 50% relative humidity (RH). All volumes tested gave quantitative results (over 94% recovered). Storage stability of target compounds was tested by loading known quantity of a reference standard mix onto 10 sorbent tubes. Sorbent tubes were immediately stored in freezer (< -25^{o}C) for 14 days. Following storage, sorbent tubes were analyzed along with sorbent tubes that were recently (less than 1 day) loaded. Results demonstrate that storage stability of target compounds was excellent with greater than 90% recovered for all compounds when comparing recently loaded tubes to stored tubes.

The air sampling apparatus (i.e., gas sampler, fittings and tubing) was tested for potential to sorb target compounds. Reference standards were introduced into the air sampling apparatus using an ATIS™ system (Supleco, Inc., Bellafonte, PA) that was connected to the gas sampler via glass tubing (178 x 6 mm diameter). The ATIS™ system uses flash vaporization to volatilize reference standards into a flowing air stream. The ATIS™ system was maintained at 110^{o}C and transfer room air at 100 mL min^{-1}. After approximately 250 mL of air transferred through the ATIS™ system, the air sampling apparatus was removed from the ATIS™ system and attached to a Teflon manifold. The Teflon manifold was supplied with a constant nitrogen gas stream maintained at ambient temperature (23 + 1.5^{o}C) and 50% relative humidity. The air sampling program used by the gas samplers during the study was similarly used in this experiment (i.e., 100 mL min^{-1} flow rate for 12 L). Recovery of standards from the air sampling apparatus was compared to reference standards loaded directly onto sorbent tubes using the ATIS™ system.

The transfer efficiency of target compounds through the air sampling apparatus was incorporated into the final emission equation through a correction factor (i.e., emission factor divided by recovery of target compound in air sampling apparatus).

Air Sampling

Air sampling of VFAs and phenolic compounds was conducted at the inlet and exhaust ducts of an environmentally controlled animal chamber along with periodic grab samples using sorbent tubes from within the animal chamber at UC-Davis swine facility. Field gas samplers (GS 301 gas sampler, SARSTEDT Inc., Newton, NC) were connected to the air handling system of the animal chamber using both quick-connect fittings and flexible tubing. Material used to attach field gas samplers to the air handling system were constructed of polypropylene, Teflon or Tygon material, respectively. Surfaces exposed to the flow path prior to the sorbent tubes were tested for their capacity to absorb target compounds (See Method Validation for detail).

All samples were collected on glass sorbent tubes (178 x 6 mm diameter) containing a multi-bed sorbent packing of Carbopack C and Carbopack X (1:2 ratio v/v) custom made by Supelco, Inc. (Bellafonte, PA). Characteristics of the each sorbent material are shown in the available spreadsheet files. Prior to use, the sorbent tubes were conditioned on a Tube Conditioner (Gerstel, Inc. Baltimore, MD) at 325^{o}C for a minimum of 2 hours with a nitrogen purge of 50-70 mL min^{-1}. Conditioned tubes were sealed with Teflon faced lined septum and end caps and stored in tube holding containers. At predetermined time intervals, duplicate samples were taken from both the inlet and exhaust ducts. Periodic grab samples were collected within the animal chamber with a field gas sampler. The air sampling program used had an initial 1.0 L purge volume (purged at 1.5 L min^{-1}) followed by sample collection at 100 mL min^{-1} for 12 L (sampling time approximately 2 hours). Field blanks were collected by exposing sorbent tubes to ambient conditions with no air flow. After each replication (total of three replication for each animal group), sorbent tubes were removed and replaced with new conditioned sorbent tubes. Sampled sorbent tubes were stored in a small cooler filled with dry ice and transported back to the lab and stored in a freezer (<-25^{o}C). Twice a week sorbent tubes were shipped overnight to Ames, Iowa in a cooler packed with dry ice. Sorbent tubes upon receipt in Ames, Iowa were inspected, verified and immediately placed into a freezer (< -25^{o}C). All samples were analyzed within 14 days of the time they were sampled in the field. Prior to analysis, all samples were allowed to equilibrate to ambient temperatures. After analysis, the sorption tubes were conditioned as previously specified.

Analytical Analysis

Reference Standards and Calibration

A stock standard solution for nine volatile fatty acids was prepared in HPLC grade water (Burdican and Jackson, Mustegon, MI). The stock solution consisted of the following compounds: acetic acid (35.04 mM), propionic acid (13.40 mM), isobutyric acid (1.08 mM), butryic acid (10.87 mM), isovaleric acid (0.92 mM), valeric acid (4.60 mM), isocapronic acid (0.06 mM), capronic acid (0.06 mM), heptanoic acid (0.06 mM). All chemicals were 99% pure or higher (GC grade) and purchased from Aldrich (Sigma-Aldrich, St. Louis, MO). The VFA reference standard solutions were typically diluted 1:10, 1:100; and 1:1000 in HPLC grade water and pH adjusted with 100 µL of concentrated formic acid (J.T. Baker, Phillipshurg, NJ). In addition, a 10 mM VFA standard mix was obtained from Supelco (Supelco, Inc) and used during the initial method validation testing procedures. A reference standard stock solution for seven aromatic compounds was prepared in methanol (Capillary GC Grade, Sigma Aldrich). The stock solution consisted of the following compounds: phenol (5.1 mM), 2-methylphenol (0.7 mM), 2-ethylphenol (6.2 mM), 3-methylphenol (0.7 mM), 4-methylphenol (50.2 mM), indole (2.6 mM), and 3-methylindole (2.5 mM). All chemicals purchased from Aldrich (Sigma-Aldrich) at greater than 99% GC grade. The aromatic reference solutions were typically diluted 1:50, 1:250, 1:500, and 1:1000 in methanol.

Calibration curves were generated using external standards loaded onto sorbent tubes using the ATIS™ system. The ATIS™ system was maintained at 110^{o}C and purged with nitrogen at 100 mL min^{-1}for a minimum total volume loading of 250 mL for each sorbent tube. The linear calibration curves for VFAs used loading rates of 0.18 nM to 7.22 nM per tube and loading rates of 0.01 to 3.94 nM per tube for phenolic compounds. Calibration curves were created and concentrations of compounds determined from the calibration curves. After the concentrations of VFA and phenolic compounds were determined for each sample, these concentrations were corrected for transfer efficiency through the air sampling equipment. Only in the first dry cow sampling event did we extrapolate the VFA calibration curve downward, because test values were lower than anticipated, and below the generated calibration curve. The instrument calibrations were subsequently updated using standard reference concentrations that bracketed sample concentrations.

Sorbent Tube Analysis

Sorbent tubes were analyzed by thermal desorption-gas chromatography-mass spectrometry (TDS-GC-MS). The TDS was a Gerstel TDSA (Gerstel, Inc., Baltimore, MD) with a 6890 GC (Agilent Technologies, Wilmington, DE) and 5973N Inert MSD (Agilent Technologies). The instrument was equipped with PTV (programmed temperature vaporizer) inlet (CIS 4, Gerstel, Inc.) and separated compounds on a 30m x 0.25mm x 0.25µm FFAP column (J&W Scientific, Inc., Wilmington, DE) using a helium gas at 1.3 mL min^{-1} constant flow. Thermal desorption (TDS) parameters were the following: splitless mode; initial temperature, 60^{o}C; final temperature, 300^{o}C; initial time 0.5 min; final hold time 3 min; ramp, 60^{o}C min^{-1}; with a transfer line temperature of 320^{o}C.

A glass bead packed inlet was used in the PTV with the following parameters: solvent vent mode; initial temperature, -30^{o}C, final temperature, 320^{o}C, initial time, 0.2 min, final time, 3 min; ramp, 12^{o}C sec^{-1}, vent flow 20 mL min^{-1}, and purge split flow 20 mL min^{-1}. This method is essentially a 20:1 split injection from TDS to analytical column. A second method used in a few early samples had similar parameters except there was a delay in initiation of purge vent flow. The delay in the purge split flow was set at 1.2 min creating what is essentially a splitless injection from the TDS to the analytical column. This second method was used to compensate for the low concentrations of the target compounds; however, due to poor reproducibility in this second method, it was abandoned for the first method.

The GC oven temperature program was: 1) initial temp, 80^{o}C hold 0.05 min; 2) ramp 10^{o}C to 220^{o}C; and 3) ramp 50^{o}C to 240^{o}C and hold 5 min. The MS transfer line and source temperatures were 240 and 150^{o}C, respectively. Mass spectrometer was operated under SIM mode using the following monitoring ions: 1)VFA compounds monitored 43, 57, 60, 73, 74, and 87, 94, 101 m/z from 3-14.1 min; 2) phenolic compounds monitored 43, 57, 60, 73, 74, and 87, 94, 101 m/z (14.1-16.0 min); and 3) indolic compounds monitored 43, 57, 60, 73, 74, and 87, 94, 101 m/z (16.0-20.0 min).

Method Validation

The reactivity of Carbopack X packing material has been shown to oxidize small alcohols to ketones and aldehydes (Kornacki et al., 2005). In our validation, we tested to see if the sorbent tubes transformed the smaller VFAs and found no evidence of transformed product.

Consequently, the Carbopack X material was considered appropriate for analysis of both VFAs and phenolic compounds.

In a previous study, Trabue et al. (2005) determined that 2 L of air (both dry and humidified) gave quantitative recovery of VFA compared to reference standards. Comparisons of 2, 4, 6 and 12 L of air showed no significant differences in recovery of VFAs from sorbent tubes (data not shown). Recovery of VFA standards from sorbent tubes challenged with 12 L of humidified air was shown to be quantitative when compared to reference standards with recoveries ranging from 94-106%. Consequently, the SSV was set at 12 L.

Storage stability of compounds on sorbent material was tested since duration of storage has been shown to significantly affect recovery of VOCs (Dettmer et al., 2000; Volden et al., 2005). A 14 day storage stability test was used to test for stability of target compounds since no sample was stored for longer than 14 days. Recovery of both VFA and phenolic compounds were quantitative with recoveries ranging from 95-106% for VFAs and 90-109% for phenolic compounds compared to reference standards. Consequently, the potential loss of target compounds during storage was considered insignificant.

Sorption of compounds on walls of tubing can lead to substantial loss of material during sampling even when compounds are below their vapor pressure saturation (Helmig et al., 2003). Flexible tubing (i.e., Tygon®) has also been shown to aborb both large and small molecules with contact time having a significant effect on total sorption (Unger et al., 2001; Bahai and Romansky, 2002). However, it should be pointed out that sorption studies with Tygon® tubing have focused on aqueous liquids with contact times of several hours. In this study, total contact time of gases in the sampling apparatus prior to sorbent tubes is estimated at less than 30 s. In general, recovery of VFAs following passage through the air sampling apparatus was quantitative ranging from 82% (pentanoic acid) to 100% (both acetic acid and propinoic acid). Overall average recovery for VFAs was 94%. Recovery of phenolic compounds from the air sampling apparatus was not quantitative with substantial losses for all compounds. Recovery of phenolic compounds ranged from 43% (phenol) to 8% (4-ethylphenol). Overall, average recovery for phenolic compounds was 19%. Consequently, sorption of phenolic compounds on surfaces of the sampling apparatus was corrected for by scaling total concentrations higher to reflect the estimated percent loss.

Method Performance

The limit of quantitation (LOQ)[1] was defined in this study as the lowest concentration level for which the relative standard deviation (RSD) was less than 30%. The LOQ for the VFAs ranged from 10.5 ng (acetic acid) to 47.4 ng (2-methylpropanoic acid), and correspond to an air concentration of 0.8 to 3.8 μg m^{-3} air, respectively, based on theoretical sampling volume of 12 L of air and correcting for transfer efficiency through the air sampling apparatus. The LOQ for VFAs based on a ppbv scale ranged from 0.26 (pentanoic acid) to 1.02 (2-methylpropanoic acid). The LOQ for phenolic compounds ranged from 0.38 ng (2-methylphenol) to 5.43 ng (4-methylphenol) and correspond to 0.02 to 2.7 μg m^{-3} air, respectively. The LOQ for phenolic compounds based on a ppbv scale ranged from 0.04 (2-methylphenol and 2-ethylphenol) to 0.59 (4-methylphenol). See Table 3 for specific listing of LOQ for each compound. Three VFA compounds (i.e. isocapronic acid, capronic acid and heptanoic acid) were not reported since these compounds were never detected on any sorbent tubes analyzed. It should also be noted that due to the large RSD values associated with both 2-methylpropanoic and 3-methylbutanoic acids these compounds were not quantifiable. The LOQs for 4-ethylphenol were assumed similar to 2-ethylphenol; however, detection of 4-ethylphenol was well below its set LOQ value. The higher LOQ values for VFA compounds compare to phenolic compounds reflect how the volatility of the VFA compounds are sensitive to the pH of the environment. The linear range of the method was defined as RSD of less than 30% and accuracy between 75-125% of predicted. The linear range for VFAs was as large as 10.5 ng to 1052 ng for acetic acid and as small as 24.8 ng to 496.5 ng for propionic acid. The linear range for phenolic compounds was as large as 5.43 ng to 496.5 ng for 4-methylphenol and 0.38 to 0.75 for 2-methylphenol**.** See Table 3 for specific listing of linear ranges for each compound.

Emissions Calculations

In almost all sample tubes (both inlet and outlet air), both VFA and phenolic compounds were detected. However, detection of all compounds was low and the majority of the detections were below the method LOQ. Calculations of emission levels of each target compound for either inlet or outlet air was preformed on only those samples that were above the method LOQ. Final emission levels were based on calculated emissions from the outlet air minus calculated emission from inlet air. If the inlet air was higher than the outlet air for a given set of samples, the calculated emission for that sample was calculated as zero emission rather then a negative

[1] The LOQ is not identical with LOD (limit of detection). The LOD is generally expressed as the smallest concentration that can be detected with reasonable certainty for a given analytical procedure.

emission. In addition, if one replicate in a sample was below the method LOQ and the other above the method LOQ, the reported value for that sample would be only for the compound above the LOQ. If both replicates in a sample were above the method LOQ, the average of the two replicates was reported for that sample.

The concentration of VFA and phenolic compounds in individual air samples was calculated by taking mass of the compound (if above method LOQ) and dividing it by the volume of air sampled (typically final volume was 12.7 liters per sorbent tube). This number would then be divided by the transfer efficiency of that compound in the sampling apparatus, which are included in the available spreadsheets. The final concentration was reported in μg m^{-3}. The final concentration number was converted to ppbv (parts per billion volume) using the following equation:

$$ppbv = [(C_1) \times (273 + T_s)]/[(12.186 \times MW_s)] \quad (1)$$

where C_1 is concentration of the target compound in μg m^{-3}; Ts is temperature at sampling in C; 12.186 is a constant used to convert compound mass to a volume measurement; and MW_s is the molecular weight of the target compound. This equation assumes pressure was constant.

AMINES

Air sampling for amines compounds was conducted at both inlet and outlet ducts of the environmentally controlled animal chamber using a sampling train. A known volume of air was drawn through a series of collection vessels ("midget impingers"), which contain sulfuric acid (0.1 N H_2SO_4). Upon reacting with the H_2SO_4, the amines in the air stream were converted to their sulfate salts. For most amines, these salts are less volatile and more stable (e.g. more resistant to oxidation and chemical decomposition) than the free amine. The collected sample was then neutralized, liberating the free amine for subsequent analysis.

The sampling train was assembled in the following order: two impingers with 15 ml of 0.1 N H_2SO_4 each, one empty impinger, one silica gel-loaded impinger, flow meter, and pump. A minimum of one method blank and one trip blank were collected with each batch. Ice was used to cool all impingers, which had at least 15 ml under the ice surface. A mass flow monitor, which was placed in line after the filter assembly was used to set the flow rates of the pump to 1.0 L of

air per minute. Sampling periods were 120 minutes. Exact start and end times for sampling were recorded. Experimental notes of all relevant monitoring parameters included locations, tube identification numbers, pump flow rates, dates, times, sampled volumes, ambient conditions etc.

The total volume of sampled dry gas was calculated by multiplying the average flow rate of the sampling pump by the total sampling time. This sample volume was adjusted to standard conditions (20 °C, 760 mm Hg or 68 °F, 29.92 in. Hg) using the following equation. Express $V_{m(std)}$ in liters (One cubic ft. = 28.316 L).

$$V_{m(std)} = V_m Y\left(\frac{T_{std}}{T_m}\right)\left[\frac{P_{bar} + \frac{\Delta H}{13.6}}{P_{std}}\right]$$

Where:

$V_{m(std)}$ = Volume of gas sample measured by the DGM, corrected to standard conditions
V_m = Volume of gas sample
T_{std} = Standard absolute temperature, 293 K
T_m = Absolute average DGM temperature, K
P_{bar} = Barometric pressure at the sampling site, mm Hg
P_{std} = Standard absolute pressure, 760 mm Hg
ΔH = Average pressure differential across the orifice meter, mm H_2O
13.6 = Specific gravity of mercury.

Samples were completely transferred from impingers to 50 ml flask bottles. Deionized water or 0.1 N H_2SO_4 was used to rinse out all interior surfaces of the two trapping solution impingers, as well as their corresponding graduated cylinders. All samples were placed on ice in a suitable cooler, and transport to the laboratory for analysis. They were stored in a refrigerator (4°C) until analysis, which was no more than 2 weeks after collection.

Ion chromatography was used for identifying and quantifying amines. Amines were separated based on affinity toward a cation-exchange resin (which provides separation from ammonia and alkali cations), and quantified based on conductivity. The Ion Chromatography (IC) 2000 system (Dionex Corporation, Sunnyvale, CA) used in the present study consists of an AS40 autosampler, GP50 gradient pump, CD25 conductivity detector, LC20 column enclosure, Cation Self-Regenerating Suppressor (CSRS ULTRA), and Chromeleon Chromatography Management Systems. The IonPac CS17 (Dionex Corporation, Sunnyvale, CA), which has a hydrophilic, carboxylate-functionalized cation exchanger, was used for analysis of amines with excellent efficiency and peak shape.

Diluted methanesulfonic acid was used as the mobile phase with a flow rate of 1 ml/min. The sample injection volume is 25 μl. The temperature of the column was controlled at 30 °C. Suitable gradient profiles for analysis of amines are given in Section 5 of the CS17 Product Manual (Dionex Corporation, Sunnyvale, CA).

A minimum of five calibration standards with five different concentrations were analyzed to get a five-point calibration curve for each sample analysis group. At least one of the calibration standards corresponded to a sample concentration at or below that necessary to meet the data quality objectives of the project. A 100 ppb amine standard was mixed into samples as spiking to check the retention time, and separation of amines from other compounds.

The concentration of each amine in the diluted impinger solution was determined by application of the ion chromatography (IC) calibration equation. The volume of each individual amine compound in the sample was calculated:

$$V_a = \frac{(N)(0.1)(24.04)(0.001)}{(FW_a)}$$

Where:
V_a = Volume of individual amine gas in the sample of gas taken from the source
N = Average concentration of amine (mg/L) in the solutions obtained from the two impingers
0.1 = Conversion factor, assuming sample in each of the two impingers was diluted to 50 mL (0.05 L)
24.04 = Liters of ideal gas per mole of substance
0.001 = Factor to convert mg/L to g/L
FW_a = Formula weight of amine analyte

The ppmV (Ca) of each amine analyte present in the gas sample was calculated:

$$C_a = \frac{V_a, L}{V_{m(std)}, L} \times 10^6$$

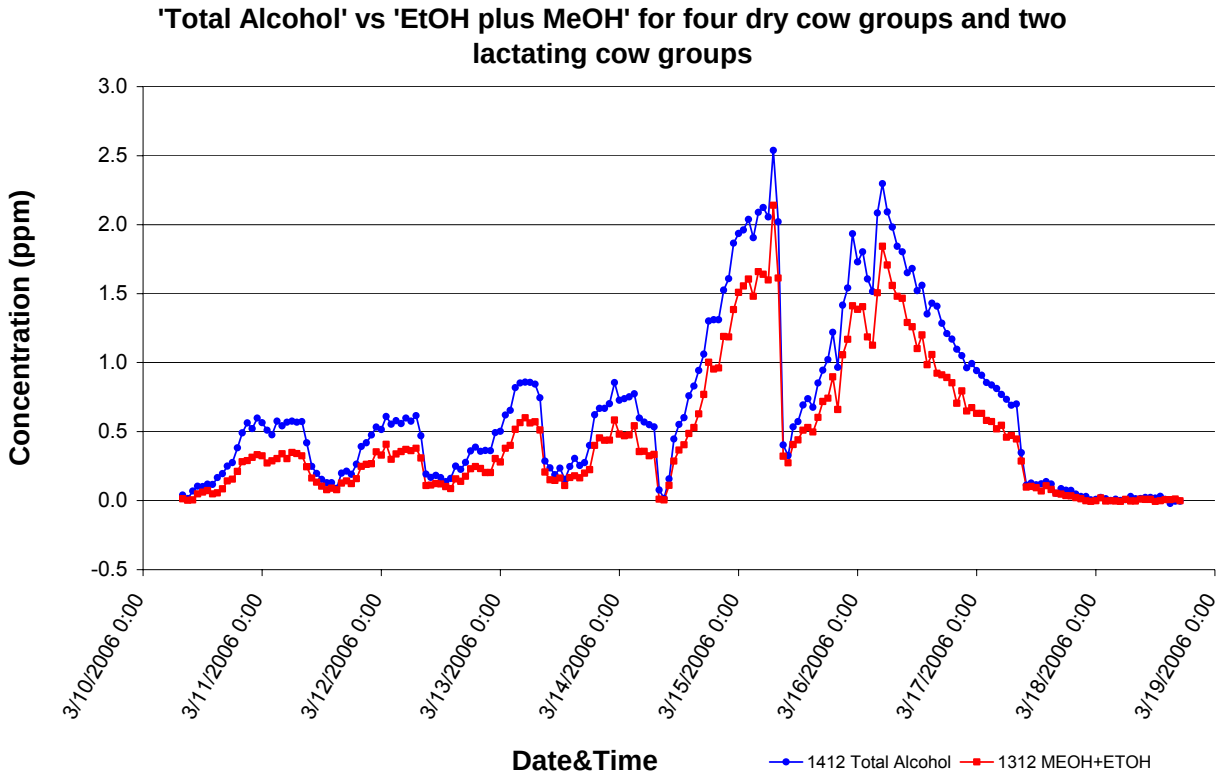
Two detailed Standard Operating Procedure (SOP) for sampling and analysis of amines are submitted and attached as part of the report package.

ALCOHOLS

Total Alcohol, Methanol and Ethanol have been analyzed by using two INNOVA Photoacoustic Field Gas-Monitors (Model 1412 and 1312) during Exp. 2. Initially, we attempted to measure these alcohols in Exp 1 but due to instrument errors with the internal alcohol filters we had to

conduct an additional experiment (Exp. 2). After substantial consultation with the manufacturer California Analytical Instruments (CAI), we replaced the faulty instrument and added a second analyzer for all subsequent testing performed in Exp 2.

The instrument has a linear response over a wide dynamic range with high stability. It can measure almost any gas, which absorbs infra-red light. By properly selecting the filters, the analyzer can selectively measure up to 5 component gases and water vapor simultaneously. The detection limit for Methanol is 0.07 ppm and for Ethanol 0.055 ppm. Both instruments were factory calibrated monthly.



Appendix figure 1: Outlet concentrations of 'total alcohols' and 'EtOH plus MeOH' for four dry cow groups and two lactating cow groups measured in parallel using two instruments (INNOVA 1412 and Innova 1312).

VFA grab sampling using sorbent tubes

Grab samples for VFAs were taken in a previous and also the present UC Davis study. Although the grab samples cannot be used to calculate an emission factors, they provide additional information about VFA concentrations in the chamber. Based on discussions by the Dairy Research Group, grab samples were used to provide additional information on the concentration variations in the chamber. (The chamber is designed to mix the air as demonstrated in previous chamber characterization studies. The proposed grab sampling should neither be considered a chamber characterization study nor a comprehensive concentration profile study.)

It is also known that conducting grab samples requires that the chamber be opened (while inlet / outlet testing is conducted from above the chamber without disturbing the inside conditions), which changes the air flow, and disturbs the cows (if present). Any grab sampling creates the risk of disturbing or altering the inlet / outlet testing that are the basis of determining emission factors. The project team minimized frequency of grab sampling the end of each sampling period (see tables 3 and 4) in the following manner:

1. We conducted grab samples for 1 set of dry and 1 set of lactating cows, at the sampling times indicated in Tables 2 and 3. This minimized the disturbance during inlet / outlet testing and reduced cost, while still meeting the request for grab samples.
2. One VFA Gerstel auto sampler was placed at the same height as samplers were placed during the previous trial. The sampling height was three feet above the ground adjacent to the fence line (out of reach of animals). Another VFA Gerstel auto sampler sampled at the chamber inlet manifold and a third sampler at the chamber outlet manifold.
3. Grab samples for acetic acid, which was the highest concentration VFA measured, ranged from below the level of quantification (LOQ) to 13.6 ppbv (parts per billion by volume). The average acetic acid concentration from all of the grab samples is approximately 3.3 ppbv. Other VFAs were much lower than this, and generally below LOQ.